Cover Page

Universiteit Leiden

Author: Duinen, Nicolette van
Title: Hereditary paragangliomas : clinical studies
Issue Date: 2012-03-07

Hereditary paragangliomas

Clinical studies

N. van Duinen

Printing: Ridderprint
ISBN: 978-90-5335-517-6

Image on cover: Hoofdingang van het gasthuis aan de Visstraat te Dordrecht omstreeks 1826. (Olieverfschilderij door C. van Duinen)

Lay-out: Nicolette van Duinen en Andreas Nasioudis
Cover lay-out: Kees-Jan van Duinen

“No one is useless in this world who lightens the burdens of another.”
Charles Dickens

Voor Jan en Kees

Hereditary paragangliomas

Clinical studies

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. ven der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 7 maart 2012
klokke 16.15 uur

door

Nicolette van Duinen
geboren 17 juni 1981 te Dordrecht

Promotiecommissie

Promotores	Prof. dr. J.A. Romijn Prof. dr. J.W.A. Smit
Co-promoter	Dr. E.P.M. van der Kleij-Corssmit
Referent	Prof. dr. J.W.M. Lenders, Universitair Medisch Centrum St. Radboud, Nijmegen
Overige leden	Prof. dr. I.P. Kema, Universitair Medisch Centrum Groningen Prof. dr. J.H. Bolk Dr. K.W. van Kralingen Dr. J.C. Jansen

Contents

Chapter 1

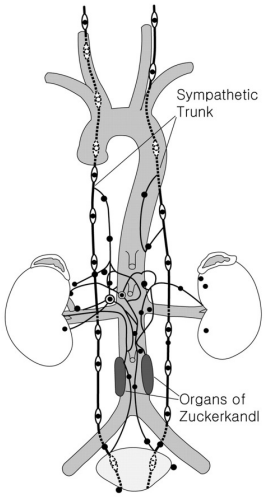
General Introduction

1 General Introduction

1.1 Introduction

Paragangliomas (PGLs) are highly vascular, neuroendocrine tumors of paraganglia, cell clusters originating from the neural crest, that have co migrated with the autonomic nervous system (1).In general, paragangliomas in the head and neck region (HNPGL; also called glomus tumors) are associated with the parasympathic system, whereas adrenal and extra-adrenal non-HNPGLs are associated with the sympathetic nervous system. Adrenal paragangliomas are also refered to as pheochromocytomas. In the medulla of the adrenal glands neural crest cells ultimately result in formation of chromaffin cells that are able to produce and secrete catecholamines. In addition, most extra-adrenal non-HNPGLs produce catecholamines as well. Although HNPGL have the potential to produce and secrete catecholamines, only a minority of these patients actually have biochemical evidence of catecholamine excess (2). PGL can occur either sporadically or hereditary, as part of a familial syndrome (MEN-2, NF1, VHL or familial PGL, due to mutations in cRET, NF1, VHL and SDH respectively) (3-9).

Figure 1: Distribution of the paraganglion system



Adapted from: J.C. Jansen, P.B. Douwes Dekker. Coutesy of AFIP, tumors of the extra-adrenal paraganglion system (including chemoreceptors), Washington DC, Armed Forces Institute of Pathology, 1974

1.2 Etiology

Paragangliomas and pheochromocytomas can occur as a consequence of a mutation in the succinate dehydrogenase (SDH) gene. The SDH gene family (SDHA, SDHB, SDHC and SDHD) encodes the four subunits of complex II of the mitochondrial electron transport chain. The nature of the germline mutations in complex II subunits predicts loss of function of the mutant variants (10). The subsequent somatic loss of the non mutant alleles in these tumors suggest that these genes function as tumor suppressor genes in the paraganglionic system (11). Although the molecular steps linking loss of complex II subunits to cellular proliferation are unknown (11), two plausible hypotheses have been proposed to explain the linkage between disruption of electron flow through mitochondrial complex II and tumorigenesis in neuro-endocrine cells. SDH contributes to the energy metabolism as a component of the tricarboxylic acid cycle, converting succinate to fumarate, and by serving as a source of electrons for mitochondrial respiration, as complex II of the electron transport chain. In the succinate accumulation hypothesis increased succinate resulting from loss of SDH function can inhibit the activity of prolyl hydroxylases (PHDs). PHDs are enzymes that are required for the degradation of HIF (hypoxia induced factor). Thus HIF becomes activated as a result of SDH loss of function (12;13). Hypoxia induced factors are transcription factors that respond to changes in available oxygen in the cellular environment, specifically hypoxia. Physiological processes regulated by HIF-1 target genes are erythropoiesis, angiogenesis and glycolysis (14). Many types of cancer have been associated with elevated levels of hypoxia-induced factor. The second hypothesis is based on the data of Lee *et al.* (Lee et al, Cancer cell 2008:155-167), who reported escape from apoptosis of sympathoadrenal progenitor cells at the end of the embryonic period. Normally, in the beginning of the embryonic period, there is a lot of nerve growth factor which inhibits apoptosis in order for the nervous system to develop. At the end of the embryonic period, nerve growth factor decreases considerably, resulting in apoptosis of a large proportion of neuronal progenitor cells. In case of mutations in the paparaganglioma genes cRET, NF1, VHL and SDH, apoptosis is inhibited, thereby resulting in formation of paragangliomas. There is an autosomal-dominant role of transmission for all PGLs with a parent of origin dependent inheritance in SDHD mutation carriers. The disease phenotype is transmitted only through the fathers, whereas mothers do not transmit the disease (15-17). This observation strongly suggests that SDHD is subject to genetic imprinting, although the exact molecular mechanism remains unknown. There is no evidence of a parent specific disease transmission in families with SDHB or SDHC mutations (3;5;11;18).

1.3 Pathology

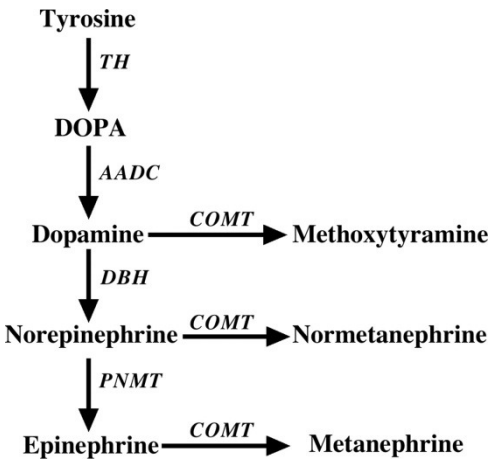
Paragangliomas are usually well demarcated and often encapsulated. Paragangliomas are characterized by its architecture, the tumor cells are arranged in distinctive cell balls, or “Zellballen”. These cell balls are separated from one another by a fibrovascular stroma. The “Zellballen” are composed of clusters of chief cells that are surrounded by supportive sustentacular cells at the periphery. The chief cells have an epitheloid appearance and vary

from ovoid to polyhydral (19;20). These chief cells, which are neuro-endocrine cells, express neuron specific enolase, serotonin, and chromogranin, and the sustentacular cells express S-100 protein (20). Membrane-bound electron-dense neurosecretory granules may be seen on electron microscopy (21;22). These neurosecretory granules vary in size, configuration and electron density of their cores (22). The sustentacular cells are spindled and are devoid of granules. Approximately 10% of HNPGLs are malignant. The histologic features of malignant paragangliomas are basically identical to those of benign tumors, but may exhibit the following features: central necrosis of Zellballen, vascular and lymphatic invasion, and the presence of mitotic spindles (23). Because there are no accepted pathological or immunohistochemical markers that distuingish malignant from benign tumors (24), malignancy is only diagnosed when metastasis to non-neuroendocrine tissue is demonstrated.

1.4 Catecholamine biosynthesis

Catecholamines are molecules that have a catechol nucleus consisting of benzene with two hydroxyl side groups plus a side-chain amine. Catecholamines include dopamine, norepinephrine and epinephrine. The L-stereoisomer of amino acid tyrosine from a dietary source or following hydroxylation of phenylalanine in the liver serves as a substrate for the initiation of catecholamine synthesis. It is converted to dihydroxyphenylalanine (dopa) by tyrosine hydroxylase (TH) and represents the rate limiting step in catecholamine biosynthesis. Tissue expression of this enzyme is largely confined to dopaminergic and noradrenergic neurons of the central nervous system, sympathetic nerves of the peripheral nervous system, chromaffin cells of the adrenal medulla and extramedullary paraganglia. The following step represents the production of dopamine by decarboxylation of dopa aromatic L-amino acid decarboxylase. Dopamine formed in neurons and chromaffin cells is translocated from the cytoplasm into vesicular storage granules. Large amounts of dopamine are also produced as an end product of catecholamine synthesis in peripheral non-neuronal cells of the gastrointestinal tract and kidneys. The dopamine formed in noradrenergic neurons and chromaffin cells is converted to norepinephrine by dopamine β-hydroxylase (DBH), an enzyme that is found only in the vesicles of cells that synthesize norepinephrine and epinephrine. In adrenal medullary chromaffin cells, norepinephrine is metabolized by the cytosolic enzyme phenylethanolamine (PNMT) to form epinephrine.
Epinehrine is then translocated into chromaffin granules, where the amine is stored awaiting release. Expression of PNMT in extra-adrenal paragangliomas is negligent, which explains the preferential production of norepinephrine by these tumors, compared to the production of both norepinephrine and epinephrine by adrenal pheochromocytomas (25). Translocation of catecholamines into vesicular granules for storage is facilitated by two vesicular monoamine transporters: VMAT1 and VMAT2 (26). Storage vesicles represent a complex functional unit that continuously maintains a highly dynamic equilibrium between passive outward leakage of catecholamines into the cytoplasm counterbalanced by VMATs- driven inward active transport.

Figure 2: Pathways of catecholamine synthesis and O-methylation

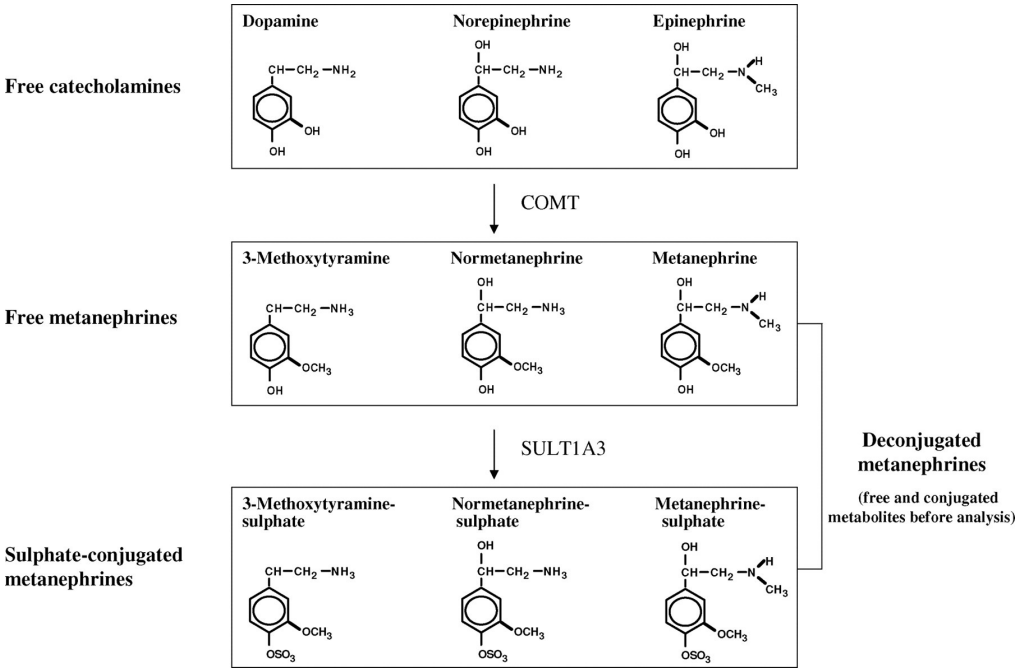


Courtesy of: Dr. G. Eisenhofer. TH, tyrosine hydroxylase; AADC, aromatic amino acid decarboxylase; DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine-N-methyltransferase; COMT, catechol-O-methyltransferase.

Catecholamines share the acid environment of the storage granule matrix with adenosine triphosphate (ATP), peptides and proteins, of which the most well known are the chromogranins. The chromogranins are ubiquitous components of secretory vesicles, and their widespread presence among endocrine tissues has led to their measurement in plasma as sensitive, albeit relatively non-specific markers of neuro-endocrine tumors, including pheochromocytomas and paragangliomas (27;28). All the free main catecholamines (dopamine, norepinephrine and epinephrine) have tightly regulated metabolism and organ-specific secretion. While dopamine is a major central neurotransmitter, its peripheral levels can be elevated in rare dopamine-secreting pheochromocytomas (29;30). The adrenal gland can secrete both epinephrine and norepinephrine direct in the circulation, of which about 90% is rapidly removed by the extra-neuronal hepatic monoamine transport system. Paraganglia exclusively secrete norepinephrine, which is a major neurotransmitter within the sympathetic nervous system (2). For norepinephrine released by sympathetic nerves ~ 90% is removed by neuronal reuptake, 5% is removed by extraneuronal uptake and 5% escapes these processes to enter the bloodstream. Catechol-O-methyltransferase (COMT) is responsible for a major pathway of catecholamine metabolism, catalyzing O-methylation of dopamine to methoxytyramine, norepinephrine to normetanephrine and epinephrine to normetanephrine. Normetanephrine and metanephrine are produced in small amounts and only at extra-neuronal locations, with the single largest source representing adrenal chromaffin cells, which account for over 90% of circulating metanephrine and 24-40% of circulating normetanephrine. Approximately 90% of the vesicular monoamine (VMA) formed in the body is produced in the liver, mainly from hepatic uptake and metabolism of circulating DHPG and MHPG. In humans, VMA and the sulphate and glucoronide

conjugates of MHPG represent the main end products of norepinephrine and epinephrine metabolism and are eliminated mainly by urinary excretion (25).

Figure 3: Pathways of metabolism of catecholamines to free and sulfate-conjugated metanephrines



Courtesy of: Dr. W.H.A. de Jong. Dopmanine, norepinephrine, and epinephrine are metabolized to free 3-MT, NMN, and MN, respectively, by the enzyme catechol-O-methyltransferase (COMT). The free MNs may than be conjugated with a sulfate group by the sulfotransferase isoenzyme 1A3 (SULT1A3).

1.5 Clinical presentation

1.5.1 Head and neck paragangliomas

Head and neck paragangliomas usually grow slowly. Only a minority has a growth rate of more than 20% (31). Because of this slow grow rate, HNPGL remain clinically silent for years. The clinical presentation of head and neck paragangliomas is dominated by local mass effects symptoms (2). Carotid body paragangliomas (CBTs) are the most common HNPGL. The most common presenting sign is a painless cervical mass (32). When a bruit over the mass is present, significant compression of the artery is present (33). Large compressive CBTs may result in cranial nerve paralysis, including vagal and hypoglossal paralysis. Symptom duration may range from 1 to 5 years prior to the diagnosis.

Vagal body paragangliomas present as painless neck masses, located behind the angle of the mandible, occasionally accompanied by dysphagia and hoarseness (34). Diagnosis and treatment of these tumors is particularly challenging because of the variable clinical behavior of these tumors. They generally grow slowly, but have the potential to surround and invade neurovascular structures, invade the skull base, and extent intracranially. Surgical excision may be associated with considerable morbidity, because of the origin of the tumor from the vagus nerve, and the surrounding cranial nerves (34). Paragangliomas of the mesotympanicum (glomus tympanicum) and jugular foramen (glomus jugulare) most commonly present as a vascular middle ear mass. These tumors often present with pulsatile tinnutis and hearing loss (35). Difficulties in speech, swallowing and airway function may be the result of dysfunction of cranial nerves traversing the jugular foramen (36). Other sites within the head and neck may represent rare locations for paragangliomas. Laryngeal paragangliomas may present with hoarseness and dysphagia secondary to mass effect rather than neurologic dysfunction (37). Other rare sites for paraganglioma include the sinonasal chambers, orbit, thyroid gland and thoracic inlet (19;38-40).

1.5.2 (Extra-)adrenal paragangliomas

Germline mutations in the SDHx genes are associated with the development of paragangliomas in diverse anatomical locations, including pheochromocytomas. Extra-adrenal paraganglionic clusters and organs include the organ of Zuckerkandl, prevertebral and paravertebral thoraco-abdominal and pelvic paraganglia, and other paraganglia in ovary, testis, vagina, urethra, prostate, bladder and liver (1). Whether anatomical locations of paragangliomas are influenced by a specific complex II mutation remains to be established (11). The clinical presentation of pheochromocyomas and extra-adrenal PGLs is highly variable and the clinical profile varies according to the type of catecholamine secreted. Most symptoms are due to elevated levels of catecholamines. Sustained hypertension is found in more than 50% of adult cases. Palpitations, headache, excess sweatiness and pallor are well-known effects of continous or paroxysmal catecholamine excess. Other complaints are anxiety, nausea, vomiting, weight loss, dyspnea, weight loss or weight gain, and generalized weakness (41;42). Direct stimulation of the tumor, physical activity, diagnostic procedures, certain drugs or food may trigger catecholamine release (43). Metabolic actions of catecholamines may lead to hyperglycemia and electrolyte disturbances as presenting symptoms, which could lead to delayed diagnosis or near-fatal complications (44).

1.5.3 Hereditary versus sporadic tumors

The major features related to predisposition for inherited disease are: familial antecedents of the disease, bilateral tumors affecting paired organs, multiple primary tumors in the same individual, tumor type and early age of onset (4;45;46). SDHD gene mutations are more prevalent in patients with HNPGL compared to SDHB or –C mutations, although this might be regionally different (4;47). Carotid and vagal PGL are more common in patients with a

familial form of the disease compared to jugular or tympanic tumors (48). Hereditary paragangliomas present earlier than sporadic cases (49). Approximately 10% of patients with HNPGL present with multiple tumors (50). In sporadic cases the incidence of multiple tumors probably represents unrecognized familial cases, because the chance of developing multiple tumors without precipitating factors is small (51). Some studies report a difference in female to male ratio between heriditary and sporadic cases. While there are more males than females with inherited HNPGL, females in the general population are more likely than males to develop sporadic tumors (4;15;52;53). SDHB mutation carriers are more likely to present with extra-adrenal non-HNPGLs and pheochromocytomas, solitary tumors, non-familial presentation, and malignant paragangliomas (49;54-58). Ninety percent of SDHB related pheochromocytomas secrete norepinephrine or dopamine (56). SDHD mutation carriers are more likely to present with HNPGLs, multiple tumors and benign paragangliomas (49;54;59). At least a third of extra-adrenal PGLs are caused by SDHD or SDHB mutations, and at least 28% of malignant pheochromocytomas are caused by SDHB germ line mutations (55;57;60). SDHC germ line mutations are characterized mainly by solitary HNPGLs and incomplete penetrance (61).

1.6 Biochemical evaluation

1.6.1 Head and neck paragangliomas

Head and neck paragangliomas have the ability to produce and secrete catecholamines (1), but only a minority (~5%) of these patients has a biochemical active tumor (2). It is unclear which test has the highest sensitivity in discovering a biochemical active head and neck paraganglioma.

1.6.2 (Extra)-adrenal paragangliomas

The biochemical detection of (extra)-adrenal PGLs depends on the demonstration of excessive amounts of catecholamines (62). Because of the episodic secretion pattern of catecholamines, false-negative test results may occur. Metanephrines on the other hand, are produced continuously within tumor cells and therefore the measurement of these metabolites is a very sensitive test for the diagnosis of pheochromocytoma (63). The recommendations of the International Symposium on Pheochromocytoma for initial diagnosis of pheochromocytoma include measurements of fractionated metanephrines in urine or plasma (or both) as available. Both tests offer high diagnostic sensitivity. However, taking test specificity in consideration, the measurement of plasma free metanephrines is preferred. None of the tests is absolutely sensitive and specific for the diagnosis of pheochromocytoma. Dietary constituents or medications can either cause direct analytical interference in measurements of catecholamines and metabolite levels or may influence the physiological processes that determine these levels (43;64). Therefore, urine and plasma samples should be collected under strict dietary regulations and after withdrawal of medication for several weeks or after changing antihypertensive medication to doxazosine.

The measurement of plasma 3-methoxytyramine has been shown to provide diagnostic value in dopamine-secreting pheochromocytoma (64).

The clonidine suppression test is intended to distinguish between pheochromocytoma and false-positive increases in plasma catecholamines and metanephrines. Clonidine acts centrally as an alpha 2-adrenergic receptor agonist that normally suppresses the release of catecholamines from neurons that does not affect the catecholamine secretion from a pheochromocytoma. Clonidine is admitted orally, and plasma catecholamines or metanephrines are measured before and after three hours after the dose. Normal plasma norepinephrine or normetanephrine levels, or their respective decrease by 50 or 40%, exclude pheochromocytoma. Because of the risk of a marked reduction in blood pressure, clonidine suppression test should not be performed in hypovolemic patients (65).
Chromogranin A (CgA) is secreted from neurosecretory vesicles, along with catecholamines (66). Plasma CgA levels are elevated in the great majority of patients with pheochromocytoma. The levels correlate with tumor mass, making CgA a useful tumor marker (28;67). Plasma CgA levels are particularly elevated in patients with malignant pheochromocytoma (68). Plasma CgA levels can also be elevated in patients with biochemically silent tumors.

1.7 Radiographical evaluation

1.7.1 Head and neck paragangliomas

The diagnostic evaluation of head and neck paragangliomas establishes the type and extent of disease, associated lesions, and assesses collateral circulation in the head and neck (69). Magnetic resonance imaging (MRI) represents the most important imaging technique for evaluation and characterization of head and neck paragangliomas. The IV administration of MR contrast media has been recommended to improve tumor detectability. MR imaging provides more diagnostic information than does CT scanning, because of the better soft tissue contrasts as compared to CT (70). CT scanning is the most useful modality for imaging of paragangliomas of the temporal bone. MRI enables multiplanar imaging of tumor extension and vessel encasement. The recommended MR pulse sequences should at least include a T1 weighted spine echo and a T2 weighted turbo spin echo sequence. T2 weight fat suppressed and contrast enhanced fat-suppressed T1 weighed sequences can offer additional diagnostic information for improved depiction of paragangliomas in the skull base region, but have not proven to be as effective as pre- and post-contrast enhanced 3D Time of Flight (TOF) MR angiography sequence (71). The MR appearance is that of a lesion exhibiting low signal intensity T1 and proton density weighted images and a high signal intensity on T2 weighted images. Paragangliomas of the head and neck are hypervascular. MRI arteriography/ venography characterize arterial and venous structures in a non-invasive fashion. MRI arteriography provides illustrations of the pertinent arterial anatomy with attendant displacement of vessels and characterization of dominant vascular supply, which may be important if surgery is being considered (72). Venography with MRI

allows for illustration of any abnormalities of the jugular bulb, internal jugular vein and pertinent dural sinuses including compression and/or thrombosis (73). In addition, paragangliomas express somatostatin receptor type 2 on their cell surface. Octreotide is a somatostatin analogue that, when coupled to a radioisotope, produces a scintigraphic image of neuroendocrine tumors expressing somatostatin type 2 receptors (SSR2) (74;75). Octreotide scintigraphy has a sensitivity of 97% and a specificity of 83% in the detection of HNPGL (76). Octreotide scintigraphy also appears to be useful in the detection of synchronous tumors and metastases in the screening of patients with hereditary PGL (76-78). Somatostatin receptor scintigraphy has also been recommended in the detection of local recurrence or residual tumor after surgery (79). ^{123}-metaiodobenzylguanidine is a radiolabeld compound that can be used to detect paragangliomas. ^{123}-metaiodobenzylguanidine provides important information if patients with unresectable tumors are suitable for treatment with ^{123}-metaiodobenzylguanidine. Ultrasound has a limited role in the evaluation of HNPGL. It may be usefull in the evaluation and follow-up of carotid body paraganglioams. Carotid body paragangliomas can be visualized as hypoechoic heterogeneous masses, which can splay the internal and external carotid arteries (80).

1.7.2 (Extra)-adrenal paragangliomas

SDH-related tumour localization studies should be carried out after biochemical diagnosis suggested existing disease in agreement with diagnostic approach to other PGLs. SDHB mutation carriers can develop biochemically silent malignant PGLs. Therefore, screening for these tumors should not be limited to biochemical tests (81). Both CT- and MRI scanning are sensitive anatomical imaging modalities, but lack specificity (82;83;83). Functional imaging techniques are able to show functional tissue. MIBG scanning is the most widely used tracer in the first line functional imaging of PGL. The sensitivity is high for primary tumors and relatively poor for metastasis (84;85). F-DOPA-PET has been confirmed to be usefull in the evaluation of sympathetic and parasympathetic PGL (86;87). ^{18}F-FDG PET/CT is the preferred technique for the localization of the primary PGL and to rule out metastases. Alternatives are ^{18}F-DOPA PET and ^{123}I-MIBG scintigraphy. For patients with known metastatic PGL, ^{18}F-FDA PET is recommended in patients with unknown genotype, ^{18}F-FDG or ^{18}F-FDA PET in SDHB mutation carriers, and ^{18}F-DOPA or ^{18}F-FDA PET in non-SDHB patients (88).

1.8 Treatment

1.8.1 Head and neck paragangliomas

Treatment of head and neck paragangliomas must be considered in relation to the tumor growth velocity, biological activity of the tumor, patient age and medical condition, tumor size and site, and potential for treatment related morbidity (89). Because most tumors grow slow, a wait-and-scan policy is often advised (31). The main treatment modalities for HNPGL are mainly surgery. Surgical success is measured by total tumor resection without

recurrence. Treatment options and recommendations vary across the spectrum of paragangliomas. A team approach is recommended for the treatment of most paragangliomas except for the very small tumors. Familial paragangliomas can be detected earlier due to screening. This allows for removal while still small. A functional HNPGL should be recognized preoperatively to allow pharmacological preparation to prevent or block the effects of acute release of catecholamines during anesthesia induction and surgery that may potentially produce lethal complications (90). α-Adrenergic blockage is the anti-hypertensive treatment of choice to prevent intraoperative hypertensive crisis. Clinically and biochemically silent paragangliomas may cause hemodynamic instability when they are manipulated during surgery (2). Surgical resection has been the mainstay of treatment of CBTs (91). Surgical morbidity has been reduced as a consequence of improvement in anesthesia and vascular surgical technique (92). The risk of arterial injury during surgery increases with increasing tumor size (92;93). Surgical resection of bilateral CBTs represents a potential problem with complete loss of the carotid chemo- and baroreceptor function (93-96). Surgical morbidity related to cranial nerve dysfunction is more common in vagal body surgery compared to carotid body resection. Nevertheless, surgical resection has traditionally been advocated. During the process of decision taking the following points should be taken into consideration: tumor size; age of the patient; monofocal or plurifocal nature of the tumor; growth rate of the tumor and 10^{th} cranial nerve loss (97;98). Excision of vagal paraganglioma almost always requires sacrifice of the vagus nerve (99). Resection of these tumors may also be associated with significant vascular injury, especially for larger tumors at the skull base (100). Given the potential for significant morbidity as a result of cranial nerve sacrifice or vascular injury, patient selection should be carefully considered prior to recommending surgery for vagal paraganglioma. A wait and scan policy is preferable to aggressive surgery for patients at risk for disabling surgical morbidity (50). Glomus jugulare tumors originate in the jugular bulb region. Symptamotology is determined by its variable growth pattern. Management of glomus jugulare tumors remains controversial. The surgical approach must be individualized to accommodate the route of tumor extension. Surgical control of the tumor can be expected in 85% of cases, tumor recurrence is seen in approximately 4.9% and subtotal resection approximates 10%. The subject of pre-embolization in managing head and neck paragangliomas remains a subject of controversy. The primary aim of pre-operative embolization is to reduce tumor vascularity which can lead to a decreased intra-operative blood loss (101). This leads to a better operative field for the surgeon, reducing the possibility of nerve or vessel damage. There is no evidence that pre-operative embolization reduces tumor dimension. To obtain the best result after selective embolization, it is recommended to perform surgery within the first 48h to minimize revascularization edema and local inflammatory response (102). An experienced vascular radiology team has to be familiar with the complexity and possible variations in head and neck vascular anatomy. The goal of surgical resection is complete tumor removal. Cervical paragangliomas are usually resected via a transcervical approach. The approach choosen for resection of a temporal paraganglioma depends on the location and extend of the tumor. Surgical resection carries a mortality rate of 3 to 15% (103). The rate of complications rises with the size of the tumor (89). The most common complication

is cranial nerve damage, observed in 18 to 50% of patients (104). Vascular complications account for a total of 9-28% with an estimated risk for stroke up to 11% (105). The role of tumor irradiation in the management of paragangliomas remains controversial. External tumor irradiation may reduce tumor growth and may provide symptomatic relieve in a selected subpopulation of patients. The indications for tumor irradiation may be large tumors, in which resection may result in considerable morbidity, poor health of the patient, after incomplete resection of the tumor with intracranial or skull base invasion (101) or in the setting of multiple HNPGLs (106). Radiotherapy judges treatment efficiency by the absence of tumor growth assessed radiographically.

1.8.2(Extra)-adrenal paragangliomas

The mainstay in the treatment of (extra)-adrenal PGLs is surgery, which can be performed conventionally or by laparascopy. To avoid catecholamine- induced complications, patients should be treated pre-opertaively with α- and β-receptor blocking mediaction (107). In patients with bilateral adrenal PGLs, laparoscopic cortical sparing adrenalectomies are considerd by some authors aimed at avoidance of gluco- and mineralcorticoid deficiency. Post-operative follow-up with bi-annual measurement of blood pressure and annual assement of plasma levels or urinary excreton rates of metanephrines should be performed.

1.9 Malignant disease

1.9.1 Head and neck paragangliomas

Approximately 10% of HNPGLs are malignant. There are no histopathological or immunohistochemical features that indicate the malignant potential of the primary tumor (108;109). In one patient series malignancy was related to pain, and a younger age at presentation (110). Malignant HNPGL have been observed more frequently in vagal PGL and in secreting tumors (111;112). Malignant HNPGL are confirmed by the presence of local metastases in cervical lymph nodes or systemic metastases (108;109;113). HNPGLs can metastasize to bones, lung and liver (113). Malignant HNPGLs occur most frequently in patients with SDHB gene mutations (46;49;114). In patients with SDHD gene mutations, malignant HNPGLs are rare and estimated to occus in less than 5 % of the patients (54;115). To date, only a single malignant HNPGL has been reported in a patient with a SDHC gene mutation (116). Because of the high risk of malignancy, patients with SDHB gene mutations should be regularly screened with three-body region MRI. This may be done with either octreotide-scintigraphy or F-DOPA PET (79;117). The primary management of patients with malignant HNPGLs should be directed towards complete surgical resection of the primary tumor and regional lymp nodes. Postoperative radiation

may be beneficial in slowing the progression of residual disease (113). Lee *et al.* reported a 5-year relative survival rate of 59.5%, 76.8% for regionally confined carcinoma and 11.8% for distant metastases (113). A 5-year survival rate of 71.2% was reported by Manolidis *et al.* was 71.2%; (112).

1.9.2 (Extra)-adrenal paragangliomas

Treatment for malignant (extra)-adrenal PGLs include surgical debulking, pharmacological control of catecholamine induced symptoms, external radiation, and systemic antineoplastic therapy. There is no effective treatment for malignant (extra)-adrenal PGLs. Surgical debulking is widely regarded as the mainstay of palliative therapy in the treatment of malignant PGL. Treatment with therapeutic dosis of ^{131}I-MIBG or combination chemotherapy may induce (partial) responses. External beam irradiation may be usefull in the treatment of local tumor complications. Catecholamine induced signs and symptoms can be treated with α-receptor blocking medication. Prognosis in confirmed malignant PGLs is difficult to predict, but is known to be poor secondary to local recurrence or widespread metastases. 5-year survival rates are only 20% to 50%, the outcome is related to tumor size (118;119).

1.10 Genetic testing

Germline mutations in the mitochondrial complex II genes (SDHB, SDHC, SDHD) cause hereditary paragangliomas. Rare reports of HNPGL occur in MEN type 2 and VHL disease (6-9). Because 30% of patients with apparently sporadic HNPGL are affected by a SDHx mutation, molecular genetic screening should be performed in all patients with HNPGL. All SDH genes should be tested in patients with single HNPGL before the age of 45 years, but only SDHB and SDHC would be recommended above this age. The development of a single SDHD-related HNPGL in an older patient without familial antecedents is extremely rare (120). It is recommendable to screen for mutations in all patients with HNPGL, irrespective of clinical indicators of germline mutations. Given the low incidence of these patients and the relatively low workload of the genetic screening, the costs of this approach should not be too high considering the benefits. The identification of germline mutations is of importance because it gives the opportunity for early diagnosis through periodical image studies in family members of the affected individual (121). First degree relatives of mutations carriers should be screened as well. Genetic classification is essential for downstream management of the patients and preemptive management of family members (45). Early detection of a familial paraganglioma allows early surgical treatment, reducing the complication rate of this operation (48). In some restricted geographic areas specific mutations due to a founder effect increase the frequency of hereditary HNPGL. All patients from those areas should be screened for those mutations first, before application of proposed testing algorithm (15;45;122). The identification of a SDHD or SDHB mutation should lead to complete investigations before surgery to detect all paragangliomas and metastases (46). The mutation positive carriers need regular clinical follow-up (45;54). here is currently no international consensus regarding intervals and specific programs. A minimum monitoring program (54) is recommended which comprises of: 1) a careful history and physical examination in association with annual measurement of blood pressure and urinary catecholamines/ metabolites, and 2) biennial imaging (neck, thorax, abdomen and pelvis).

1.11 Outline of this thesis

Part I: High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands

Worldwide, an estimated 10% to 50% of head and neck paraganglioma cases are hereditary, although in the Netherlands this percentage may be higher due to founder mutations. The hereditary paraganglioma syndrome can be caused by mutations in genes encoding subunits or co-factors of succinate dehydrogenase (SDH), an enzyme complex bound to the inner membrane of the mitochondria. In the Netherlands, several founder mutations, characterized by a specific DNA haplotype that is common to all carriers of the mutation, have been described in SDHB, SDHC and SDHAF2. In **chapter 2**, we studied mutation frequencies of different genes y in 1045 paraganglioma and pheochromocytoma patients and their relatives who tested positive for a mutation in SDHB, SDHC, SDHD or SDHAF2. Results were obtained from the Leiden University Medical Centre (LUMC), a dedicated referral center for paragangliomas and the national referral laboratory for SDH mutations in the Netherlands. As almost all Dutch paraganglioma patient samples are analysed in the LUMC, the results represent the actual prevalence of mutations in genes encoding subunits of the SDH complex in the Netherlands.

Part II: the measurement and clinical relevance of 3-methoxytyramine excretion in HNPGL patients

Head and neck paragangliomas can be positive for tyrosine hydroxylase, the enzyme which converts tyrosine to L- dihydroxyphenylalanine (L-dopa), the precursor of dopamine. This observation suggests that even biochemically inactive paragangliomas may have the enzymatic ability to synthesize catecholamines. Interestingly, dopamine functions as a neurotransmitter in HNPGLs. It is unclear to which extent dopamine production is present in HNPGL in general. Dopamine is converted to 3-methoxytyramine by the enzyme catechol-O-methyltransferase. Urinary excretion rates of 3-methoxytyramine (3MT) have not been assessed in paraganglioma patients. In **chapter 3** we studies the prevalence of increased urinary 3MT excretion rates in HNPGL patients and the clinical, biochemical and radiological characteristics of HNPGL patients with increased 3MT excretion by comparing patients with and without increased urinary excretion of 3MT

It is unknown whether plasma catecholamine levels, including 3MT, are more sensitive parameters of biochemical activity of HNPGL than urinary excretion rates. For the diagnosis of pheochromocytoma, the measurement of plasma free metanephrine levels is the optimal biochemical test with the highest sensitivity and specificity. Therefore, in **chapter 4** we studied whether plasma free metanephrines and 3MT levels are more sensitive tests to detect biochemical activity of HNPGL than urinary excretion rates of catecholamines and 3MT.

Part III: A small percentage of head and neck paragangliomas secrete chromogranin A

Chromogranin A (CgA) is a secretory protein from neuroendocrine cells that mediates chromaffin granule biogenesis, necessary for catecholamine storage. CgA is secreted from neurosecretory vesicles, along with catecholamines. In accordance with these biological concepts, plasma CgA is a usefull tumor marker in patients with pheochromocytoma. In **chapter 5** we evaluated the prevalence of increased CgA levels in patients with hereditary HNPGL and identified a possible role of CgA in patients with biochemically silent tumors.**Part IV: Pheochromocytomas detected by biochemical screening in predisposed subjects have a different clinical presentation compared to patients detected by signs and symptoms**

Pheochromocytomas are rare neuroendocrine tumors derived from chromaffin tissue within the adrenal medulla. In 12-24% of cases of an apparently sporadic presentation, a pheochromocytoma is caused germline mutations in the von Hippel-Lindau gene (VHL), the RET gene (leading to MEN2), the neurofibromatosis type I gene (NF1) or one of the SDH genes encoding for subunits B, D and C of mitochondrial succinate dehydrogenase.
Sporadic pheochromocytomas are usually identified by signs and symptoms, including paroxysms of headache, sweating, palpitations and hypertension resulting from the release of catecholamines from the tumor. However, a substantial proportion of patients with pheochromocytomas do not have signs and/or symptoms, which carries the risk of unexpected life-threatening catecholamine crises. Therefore, the advice is to screen patients with a known hereditary predisposition for the development of pheochromocytomas at regular intervals by measurement of plasma levels and/or urinary catecholamine excretion rates. In **chapter 6** we describe the results of a retrospective study comparing the data of patients with pheochromocytomas detected by signs and symptoms and of patients with pheochromocytomas, detected by biochemical screening in hereditary syndromes. We compared signs and symptoms, biochemical parameters, (peri)operative outcome and long-term results between these groups.

Part V: Patients with bilateral carotid body tumors are at risk for developing sleep disordered breathing

Most frequently, paragangliomes develop in the carotid bodies, which can be surgically removed. These carotid bodies have important peripheral chemoreceptor functions for sensing arterial oxygen concentrations and, to a lesser extent, arterial carbon dioxide concentrations. Accordingly, bilateral resection of carotid body paragangliomas abolishes the ventilatory responsiveness to hypoxia. Moreover, after bilateral resection of carotid body paragangliomas, ventilatory CO2 sensitivity decreases, resulting in increased resting end-tidal CO2 pressure (PetCO2) values. In turn, these high resting PetCO2 values are associated with irregular breathing, periodic breathing and central sleep apnea. Therefore, the question arises whether these patients are prone to develop sleep disordered breathing

after bilateral resection of carotid body paragangliomas. At present, it is unknown whether carotid body paragangliomas *per se* cause dysfunction of peripheral chemoreceptor function and if patients with bilateral carotid body paragangliomas have altered chemoreceptor responses. In **chapter 7** we evaluated the presence of sleep disordered breathing in patients with bilateral carotid body paragangliomas.

References

1 Zak FG, Lawson W. The paraganglionic chemoreceptor system. (1982) 1sted. Springer- Verlag New York Inc.

2 Erickson D, Kudva YC, Ebersold MJ, et al. (2001) Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. J Clin Endocrinol Metab, 86, 5210-6.

3 Astuti D, Latif F, Dallol A, et al. (2001) Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet, 69, 49-54.

4 Badenhop RF, Jansen JC, Fagan PA, et al. (2004) The prevalence of SDHB, SDHC, and SDHD mutations in patients with head and neck paraganglioma and association of mutations with clinical features. J Med Genet, 41, e99.

5 Baysal BE, Willett-Brozick JE, Filho PA, et al. (2004) An Alu-mediated partial SDHC deletion causes familial and sporadic paraganglioma. J Med Genet, 41, 703-9.

6 Hull MT, Roth LM, Glover JL, et al. (1982) Metastatic carotid body paraganglioma in von Hippel-Lindau disease. An electron microscopic study. Arch Pathol Lab Med, 106, 235-9.

7 Kennedy DW, Nager GT. (1986) Glomus tumor and multiple endocrine neoplasia. Otolaryngol Head Neck Surg, 94, 644-8.

8 Schimke RN, Collins DL, Rothberg PG. (1998) Functioning carotid paraganglioma in the von Hippel-Lindau syndrome. Am J Med Genet, 80, 533-4.

9 Zanelli M, van der Walt JD. (1996) Carotid body paraganglioma in von Hippel-Lindau disease: a rare association. Histopathology, 29, 178-81.

10 Baysal BE, Rubinstein WS, Taschner PE. (2001) Phenotypic dichotomy in mitochondrial complex II genetic disorders. J Mol Med, 79, 495-503.

11 Baysal BE. (2002) Hereditary paraganglioma targets diverse paraganglia. J Med Genet, 39, 617-22.

12 Selak MA, Armour SM, MacKenzie ED, et al. (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell, 7, 77-85.

13 Qin Y, Buddavarapu K, Dahia PL. (2009) Pheochromocytomas: from genetic diversity to new paradigms. Horm Metab Res, 41, 664-71.

14 Jones A, Fujiyama C, Blanche C, et al. (2001) Relation of vascular endothelial growth factor production to expression and regulation of hypoxia-inducible factor-1 alpha and hypoxia-inducible factor-2 alpha in human bladder tumors and cell lines. Clin Cancer Res, 7, 1263-72.

15 van der Mey AG, Maaswinkel-Mooy PD, Cornelisse CJ, et al. (1989) Genomic imprinting in hereditary glomus tumours: evidence for new genetic theory. Lancet, 2, 1291-4.

16 Heutink P, van der Mey AG, Sandkuijl LA, et al. (1992) A gene subject to genomic imprinting and responsible for hereditary paragangliomas maps to chromosome 11q23-qter. Hum Mol Genet, 1, 7-10.

17 Baysal BE, Farr JE, Rubinstein WS, et al. (1997) Fine mapping of an imprinted gene for familial nonchromaffin paragangliomas, on chromosome 11q23. Am J Hum Genet, 60, 121-32.

18 Niemann S, Steinberger D, Muller U. (1999) PGL3, a third, not maternally imprinted locus in autosomal dominant paraganglioma. Neurogenetics, 2, 167-70.

19 Lack EE, Cubilla AL, Woodruff JM, et al. (1977) Paragangliomas of the head and neck region: a clinical study of 69 patients. Cancer, 39, 397-409.

20 Kliewer KE, Wen DR, Cancilla PA, et al. (1989) Paragangliomas: assessment of prognosis by histologic, immunohistochemical, and ultrastructural techniques. Hum Pathol, 20, 29-39.

21 Sennaroglu L, Sungur A. (2002) Histopathology of paragangliomas. Otol Neurotol, 23, 104-5.

22 Warren WH, Lee I, Gould VE, et al. (1985) Paragangliomas of the head and neck: ultrastructural and immunohistochemical analysis. Ultrastruct Pathol, 8, 333-43.

23 Lack EE, Cubilla AL, Woodruff JM. (1979) Paragangliomas of the head and neck region. A pathologic study of tumors from 71 patients. Hum Pathol, 10, 191-218.

24 Granger JK, Houn HY. (1990) Head and neck paragangliomas: a clinicopathologic study with DNA flow cytometric analysis. South Med J, 83, 1407-12.

25 Eisenhofer G, Kopin IJ, Goldstein DS. (2004) Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacol Rev, 56, 331-49.

26 Peter D, Liu Y, Sternini C, et al. (1995) Differential expression of two vesicular monoamine transporters. J Neurosci, 15, 6179-88.
27 Nobels FR, Kwekkeboom DJ, Coopmans W, et al. (1997) Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. J Clin Endocrinol Metab, 82, 2622-8.
28 Bilek R, Safarik L, Ciprova V, et al. (2008) Chromogranin A, a member of neuroendocrine secretory proteins as a selective marker for laboratory diagnosis of pheochromocytoma. Physiol Res, 57 Suppl 1, S171-S179.
29 Eisenhofer G, Goldstein DS, Sullivan P, et al. (2005) Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. J Clin Endocrinol Metab, 90, 2068-75.
30 Proye C, Fossati P, Fontaine P, et al. (1986) Dopamine-secreting pheochromocytoma: an unrecognized entity? Classification of pheochromocytomas according to their type of secretion. Surgery, 100, 1154-62.
31 Jansen JC, van den BR, Kuiper A, et al. (2000) Estimation of growth rate in patients with head and neck paragangliomas influences the treatment proposal. Cancer, 88, 2811-6.
32 Gardner P, Dalsing M, Weisberger E, et al. (1996) Carotid body tumors, inheritance, and a high incidence of associated cervical paragangliomas. Am J Surg, 172, 196-9.
33 Hodge KM, Byers RM, Peters LJ. (1988) Paragangliomas of the head and neck. Arch Otolaryngol Head Neck Surg, 114, 872-7.
34 Netterville JL, Jackson CG, Miller FR, et al. (1998) Vagal paraganglioma: a review of 46 patients treated during a 20-year period. Arch Otolaryngol Head Neck Surg, 124, 1133-40.
35 Effat KG, Karam M. (2004) Jugulotympanic paraganglioma (glomus tumour) presenting with recurrent epistaxis. J Laryngol Otol, 118, 153-5.
36 Jackson CG. (2001) Glomus tympanicum and glomus jugulare tumors. Otolaryngol Clin North Am, 34, 941-70, vii.
37 Myssiorek D, Rinaldo A, Barnes L, et al. (2004) Laryngeal paraganglioma: an updated critical review. Acta Otolaryngol, 124, 995-9.
38 Myssiorek D, Halaas Y, Silver C. (2001) Laryngeal and sinonasal paragangliomas. Otolaryngol Clin North Am, 34, 971-82, vii.
39 Kuhn JA, Aronoff BL. (1989) Nasal and nasopharyngeal paraganglioma. J Surg Oncol, 40, 38-45.
40 LaGuette J, Matias-Guiu X, Rosai J. (1997) Thyroid paraganglioma: a clinicopathologic and immunohistochemical study of three cases. Am J Surg Pathol, 21, 748-53.
41 Werbel SS, Ober KP. (1995) Pheochromocytoma. Update on diagnosis, localization, and management. Med Clin North Am, 79, 131-53.
42 Kebebew E, Duh QY. (1998) Benign and malignant pheochromocytoma: diagnosis, treatment, and follow-Up. Surg Oncol Clin N Am, 7, 765-89.
43 Lenders JW, Eisenhofer G, Mannelli M, et al. (2005) Phaeochromocytoma. Lancet, 366, 665-75.
44 La Batide-Alanore A, Chatellier G, Plouin PF. (2003) Diabetes as a marker of pheochromocytoma in hypertensive patients. J Hypertens, 21, 1703-7.
45 Neumann HP, Erlic Z, Boedeker CC, et al. (2009) Clinical predictors for germline mutations in head and neck paraganglioma patients: cost reduction strategy in genetic diagnostic process as fall-out. Cancer Res, 69, 3650-6.
46 Burnichon N, Rohmer V, Amar L, et al. (2009) The succinate dehydrogenase genetic testing in a large prospective series of patients with paragangliomas. J Clin Endocrinol Metab, 94, 2817-27.
47 Lima J, Feijao T, Ferreira da SA, et al. (2007) High frequency of germline succinate dehydrogenase mutations in sporadic cervical paragangliomas in northern Spain: mitochondrial succinate dehydrogenase structure-function relationships and clinical-pathological correlations. J Clin Endocrinol Metab, 92, 4853-64.
48 Hermsen MA, Sevilla MA, Llorente JL, et al. (2010) Relevance of germline mutation screening in both familial and sporadic head and neck paraganglioma for early diagnosis and clinical management. Cell Oncol, 32, 275-83.
49 Neumann HP, Pawlu C, Peczkowska M, et al. (2004) Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA, 292, 943-51.

50 Pellitteri PK, Rinaldo A, Myssiorek D, et al. (2004) Paragangliomas of the head and neck. Oral Oncol, 40, 563-75.

51 van der Mey AG, Jansen JC, van Baalen JM. (2001) Management of carotid body tumors. Otolaryngol Clin North Am, 34, 907-24, vi.

52 Grufferman S, Gillman MW, Pasternak LR, et al. (1980) Familial carotid body tumors: case report and epidemiologic review. Cancer, 46, 2116-22.

53 Drovdlic CM, Myers EN, Peters JA, et al. (2001) Proportion of heritable paraganglioma cases and associated clinical characteristics. Laryngoscope, 111, 1822-7.

54 Benn DE, Gimenez-Roqueplo AP, Reilly JR, et al. (2006) Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. J Clin Endocrinol Metab, 91, 827-36.

55 Amar L, Bertherat J, Baudin E, et al. (2005) Genetic testing in pheochromocytoma or functional paraganglioma. J Clin Oncol, 23, 8812-8.

56 Timmers HJ, Kozupa A, Eisenhofer G, et al. (2007) Clinical presentations, biochemical phenotypes, and genotype-phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. J Clin Endocrinol Metab, 92, 779-86.

57 Brouwers FM, Eisenhofer G, Tao JJ, et al. (2006) High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. J Clin Endocrinol Metab, 91, 4505-9.

58 Ricketts CJ, Forman JR, Rattenberry E, et al. (2010) Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. Hum Mutat, 31, 41-51.

59 Astrom K, Cohen JE, Willett-Brozick JE, et al. (2003) Altitude is a phenotypic modifier in hereditary paraganglioma type 1: evidence for an oxygen-sensing defect. Hum Genet, 113, 228-37.

60 Neumann HP, Bausch B, McWhinney SR, et al. (2002) Germ-line mutations in nonsyndromic pheochromocytoma. N Engl J Med, 346, 1459-66.

61 Schiavi F, Boedeker CC, Bausch B, et al. (2005) Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. JAMA, 294, 2057-63.

62 Lenders JW, Eisenhofer G, Armando I, et al. (1993) Determination of metanephrines in plasma by liquid chromatography with electrochemical detection. Clin Chem, 39, 97-103.

63 Lenders JW, Pacak K, Walther MM, et al. (2002) Biochemical diagnosis of pheochromocytoma: which test is best? JAMA, 287, 1427-34.

64 Eisenhofer G, Goldstein DS, Sullivan P, et al. (2005) Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. J Clin Endocrinol Metab, 90, 2068-75.

65 Eisenhofer G, Goldstein DS, Walther MM, et al. (2003) Biochemical diagnosis of pheochromocytoma: how to distinguish true- from false-positive test results. J Clin Endocrinol Metab, 88, 2656-66.

66 Montesinos MS, Machado JD, Camacho M, et al. (2008) The crucial role of chromogranins in storage and exocytosis revealed using chromaffin cells from chromogranin A null mouse. J Neurosci, 28, 3350-8.

67 d'Herbomez M, Gouze V, Huglo D, et al. (2001) Chromogranin A assay and (131)I-MIBG scintigraphy for diagnosis and follow-up of pheochromocytoma. J Nucl Med, 42, 993-7.

68 Rao F, Keiser HR, O'Connor DT. (2000) Malignant pheochromocytoma. Chromaffin granule transmitters and response to treatment. Hypertension, 36, 1045-52.

69 Jackson CG, Harris PF, Glasscock ME, III, et al. (1990) Diagnosis and management of paragangliomas of the skull base. Am J Surg, 159, 389-93.

70 Olsen WL, Dillon WP, Kelly WM, et al. (1987) MR imaging of paragangliomas. AJR Am J Roentgenol, 148, 201-4.

71 van den Berg R, Verbist BM, Mertens BJ, et al. (2004) Head and neck paragangliomas: improved tumor detection using contrast-enhanced 3D time-of-flight MR angiography as compared with fat-suppressed MR imaging techniques. AJNR Am J Neuroradiol, 25, 863-70.

72 Arnold SM, Strecker R, Scheffler K, et al. (2003) Dynamic contrast enhancement of paragangliomas of the head and neck: evaluation with time-resolved 2D MR projection angiography. Eur Radiol, 13, 1608-11.

73 Vogl T, Bruning R, Schedel H, et al. (1989) Paragangliomas of the jugular bulb and carotid body: MR imaging with short sequences and Gd-DTPA enhancement. AJR Am J Roentgenol, 153, 583-7.

74 Lamberts SW, Reubi JC, Krenning EP. (1993) Validation of somatostatin receptor scintigraphy in the localization of neuroendocrine tumors. Acta Oncol, 32, 167-70.
75 Krenning EP, Kwekkeboom DJ, Bakker WH, et al. (1993) Somatostatin receptor scintigraphy with [111In-DTPA-D-Phe1]- and [123I-Tyr3]-octreotide: the Rotterdam experience with more than 1000 patients. Eur J Nucl Med, 20, 716-31.
76 Bustillo A, Telischi F, Weed D, et al. (2004) Octreotide scintigraphy in the head and neck. Laryngoscope, 114, 434-40.
77 Myssiorek D, Tronco G. (2005) 111Indium pentetreotide imaging in the evaluation of head and neck tumors. Laryngoscope, 115, 1707-16.
78 Duet M, Sauvaget E, Petelle B, et al. (2003) Clinical impact of somatostatin receptor scintigraphy in the management of paragangliomas of the head and neck. J Nucl Med, 44, 1767-74.
79 Kwekkeboom DJ, van UH, Pauw BK, et al. (1993) Octreotide scintigraphy for the detection of paragangliomas. J Nucl Med, 34, 873-8.
80 Gritzmann N, Herold C, Haller J, et al. (1987) Duplex sonography of tumors of the carotid body. Cardiovasc Intervent Radiol, 10, 280-4.
81 Timmers HJ, Pacak K, Huynh TT, et al. (2008) Biochemically silent abdominal paragangliomas in patients with mutations in the succinate dehydrogenase subunit B gene. J Clin Endocrinol Metab, 93, 4826-32.
82 Pacak K, Linehan WM, Eisenhofer G, et al. (2001) Recent advances in genetics, diagnosis, localization, and treatment of pheochromocytoma. Ann Intern Med, 134, 315-29.
83 Ilias I, Pacak K. (2004) Current approaches and recommended algorithm for the diagnostic localization of pheochromocytoma. J Clin Endocrinol Metab, 89, 479-91.
84 Timmers HJ, Kozupa A, Chen CC, et al. (2007) Superiority of fluorodeoxyglucose positron emission tomography to other functional imaging techniques in the evaluation of metastatic SDHB-associated pheochromocytoma and paraganglioma. J Clin Oncol, 25, 2262-9.
85 van der Harst E, De Herder WW, Bruining HA, et al. (2001) [(123)I]metaiodobenzylguanidine and [(111)In]octreotide uptake in begnign and malignant pheochromocytomas. J Clin Endocrinol Metab, 86, 685-93.
86 Hoegerle S, Nitzsche E, Altehoefer C, et al. (2002) Pheochromocytomas: detection with 18F DOPA whole body PET--initial results. Radiology, 222, 507-12.
87 Timmers HJ, Carrasquillo JA, Whatley M, et al. (2007) Usefulness of standardized uptake values for distinguishing adrenal glands with pheochromocytoma from normal adrenal glands by use of 6-18F-fluorodopamine PET. J Nucl Med, 48, 1940-4.
88 Timmers HJ, Chen CC, Carrasquillo JA, et al. (2009) Comparison of 18F-fluoro-L-DOPA, 18F-fluoro-deoxyglucose, and 18F-fluorodopamine PET and 123I-MIBG scintigraphy in the localization of pheochromocytoma and paraganglioma. J Clin Endocrinol Metab, 94, 4757-67.
89 Lim JY, Kim J, Kim SH, et al. (2010) Surgical treatment of carotid body paragangliomas: outcomes and complications according to the shamblin classification. Clin Exp Otorhinolaryngol, 3, 91-5.
90 van Houtum WH, Corssmit EP, Douwes Dekker PB, et al. (2005) Increased prevalence of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with SDHD-linked head and neck paragangliomas. Eur J Endocrinol, 152, 87-94.
91 Kohler HF, Carvalho AL, Mattos Granja NV, et al. (2004) Surgical treatment of paragangliomas of the carotid bifurcation: results of 36 patients. Head Neck, 26, 1058-63.
92 Hallett JW, Jr., Nora JD, Hollier LH, et al. (1988) Trends in neurovascular complications of surgical management for carotid body and cervical paragangliomas: a fifty-year experience with 153 tumors. J Vasc Surg, 7, 284-91.
93 Netterville JL, Reilly KM, Robertson D, et al. (1995) Carotid body tumors: a review of 30 patients with 46 tumors. Laryngoscope, 105, 115-26.
94 Timmers HJ, Karemaker JM, Wieling W, et al. (2003) Baroreflex control of muscle sympathetic nerve activity after carotid body tumor resection. Hypertension, 42, 143-9.
95 Timmers HJ, Karemaker JM, Wieling W, et al. (2003) Baroreflex and chemoreflex function after bilateral carotid body tumor resection. J Hypertens, 21, 591-9.

96 Dahan A, Nieuwenhuijs D, Teppema L. (2007) Plasticity of central chemoreceptors: effect of bilateral carotid body resection on central CO2 sensitivity. PLoS Med, 4, e239.
97 Zanoletti E, Mazzoni A. (2006) Vagal paraganglioma. Skull Base, 16, 161-7.
98 Lozano FS, Gomez JL, Mondillo MC, et al. (2008) Surgery of vagal paragangliomas: six patients and review of literature. Surg Oncol, 17, 281-7.
99 Woods CI, Strasnick B, Jackson CG. (1993) Surgery for glomus tumors: the Otology Group experience. Laryngoscope, 103, 65-70.
100 Biller HF, Lawson W, Som P, et al. (1989) Glomus vagale tumors. Ann Otol Rhinol Laryngol, 98, 21-6.
101 Antonitsis P, Saratzis N, Velissaris I, et al. (2006) Management of cervical paragangliomas: review of a 15-year experience. Langenbecks Arch Surg, 391, 396-402.
102 Kafie FE, Freischlag JA. (2001) Carotid body tumors: the role of preoperative embolization. Ann Vasc Surg, 15, 237-42.
103 Patetsios P, Gable DR, Garrett WV, et al. (2002) Management of carotid body paragangliomas and review of a 30-year experience. Ann Vasc Surg, 16, 331-8.
104 Anand VK, Alemar GO, Sanders TS. (1995) Management of the internal carotid artery during carotid body tumor surgery. Laryngoscope, 105, 231-5.
105 Luna-Ortiz K, Rascon-Ortiz M, Villavicencio-Valencia V, et al. (2005) Carotid body tumors: review of a 20-year experience. Oral Oncol, 41, 56-61.
106 Wang SJ, Wang MB, Barauskas TM, et al. (2000) Surgical management of carotid body tumors. Otolaryngol Head Neck Surg, 123, 202-6.
107 Pacak K, Eisenhofer G, Ahlman H, et al. (2007) Pheochromocytoma: recommendations for clinical practice from the First International Symposium. October 2005. Nat Clin Pract Endocrinol Metab, 3, 92-102.
108 Boedeker CC, Ridder GJ, Schipper J. (2005) Paragangliomas of the head and neck: diagnosis and treatment. Fam Cancer, 4, 55-9.
109 Persky MS, Setton A, Niimi Y, et al. (2002) Combined endovascular and surgical treatment of head and neck paragangliomas--a team approach. Head Neck, 24, 423-31.
110 Chapman DB, Lippert D, Geer CP, et al. (2010) Clinical, histopathologic, and radiographic indicators of malignancy in head and neck paragangliomas. Otolaryngol Head Neck Surg, 143, 531-7.
111 Baysal BE. (2008) Clinical and molecular progress in hereditary paraganglioma. J Med Genet, 45, 689-94.
112 Manolidis S, Shohet JA, Jackson CG, et al. (1999) Malignant glomus tumors. Laryngoscope, 109, 30-4.
113 Lee JH, Barich F, Karnell LH, et al. (2002) National Cancer Data Base report on malignant paragangliomas of the head and neck. Cancer, 94, 730-7.
114 Boedeker CC, Neumann HP, Maier W, et al. (2007) Malignant head and neck paragangliomas in SDHB mutation carriers. Otolaryngol Head Neck Surg, 137, 126-9.
115 Havekes B, Corssmit EP, Jansen JC, et al. (2007) Malignant paragangliomas associated with mutations in the succinate dehydrogenase D gene. J Clin Endocrinol Metab, 92, 1245-8.
116 Niemann S, Muller U, Engelhardt D, et al. (2003) Autosomal dominant malignant and catecholamine-producing paraganglioma caused by a splice donor site mutation in SDHC. Hum Genet, 113, 92-4.
117 Hoegerle S, Ghanem N, Altehoefer C, et al. (2003) 18F-DOPA positron emission tomography for the detection of glomus tumours. Eur J Nucl Med Mol Imaging, 30, 689-94.
118 Scholz T, Eisenhofer G, Pacak K, et al. (2007) Clinical review: Current treatment of malignant pheochromocytoma. J Clin Endocrinol Metab, 92, 1217-25.
119 Edstrom EE, Hjelm Skog AL, Hoog A, et al. (2003) The management of benign and malignant pheochromocytoma and abdominal paraganglioma. Eur J Surg Oncol, 29, 278-83.
120 Cascon A, Lopez-Jimenez E, Landa I, et al. (2009) Rationalization of genetic testing in patients with apparently sporadic pheochromocytoma/paraganglioma. Horm Metab Res, 41, 672-5.
121 van Gils AP, van der Mey AG, Hoogma RP, et al. (1992) MRI screening of kindred at risk of developing paragangliomas: support for genomic imprinting in hereditary glomus tumours. Br J Cancer, 65, 903-7.
122 Taschner PE, Jansen JC, Baysal BE, et al. (2001) Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes Cancer, 31, 274-81.

Chapter 2

High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands

Erik F Hensen[1,2], Nicolette van Duinen[3], Jeroen C Jansen[1], Eleonora PM Corssmit[3], Carli MJ Tops[4] Johannes A Romijn[3], Annette HJT Vriends[4], Andel GL van der Mey[1], Cees J Cornelisse[5], Peter Devilee[4,5], Jean-Pierre Bayley[4]

[1] Department of Otolaryngology, Leiden University Medical Center, Leiden, the Netherlands

[2] Department of Otolaryngology, VU University Medical Center Amsterdam, the Netherlands

[3] Department of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands

[4] Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands

[5] Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands

Clinical Genetics 2011 Feb; 23

Abstract

Context: Mutations in four genes encoding subunits or co-factors of succinate dehydrogenase cause hereditary paraganglioma and pheochromocytoma syndromes. Mutations in SDHB and SDHD are generally the most common, whereas mutations in SDHC and SDHAF2 are far less frequently observed.

Methods: 1045 DNA samples from Dutch paraganglioma and pheochromocytoma patients and their relatives were analyzed for mutations of SDHB, SDHC, SDHD or SDHAF2.

Results: Mutations in these genes were identified in 690 cases, 239 of which were index cases. The vast majority of mutation carriers had a mutation in SDHD (87.1%). The second most commonly affected gene was SDHAF2 (6.7%). Mutations in SDHB were found in only 5.9% of samples, whereas SDHC mutations were found in 0.3%. Remarkably, 69.1% of all carriers of a mutation in an SDH gene in the Netherlands can be attributed to a single founder mutation in SDHD, c.274G>T, p.Asp92Tyr. Moreover, 88.8% of all SDH mutation carriers carry one of just six Dutch founder mutations in SDHB, SDHD and SDHAF2.

Conclusion: The dominance of SDHD mutations is unique to the Netherlands, contrasting with the higher prevalence of SDHB mutations found elsewhere. In addition, we found that most SDH-mutation related paragangliomas-pheochromocytomas in the Netherlands can be explained by only 6 founder mutations in SDHAF2, SDHB and SDHD. The findings underline the regional differences in the SDH mutation spectrum, differences that should be taken into account in the development of effective screening protocols. The results demonstrate the crucial role that demographic factors play in the frequency of gene mutations.

Introduction

Mutations in genes encoding subunits or co-factors of succinate dehydrogenase (SDH), an enzyme complex bound to the inner membrane of the mitochondria, are an important cause of hereditary paraganglioma syndrome (1-3). SDH plays an important dual role as complex II in the electron transport chain and as an enzyme of the TCA cycle, catalyzing the oxidation of succinate to fumarate. It consists of 4 subunits: a flavoprotein (SDHA), and iron-sulfur protein (SDHB), which together make up the catalytic domain, and SDHC and SDHD, both transmembrane proteins. In addition to the SDHB, SDHC and SDHD genes, an additional SDH-related paraganglioma tumor suppressor was recently identified (4). An important co-factor in SDH stability and functionality, SDHAF2 resides as a soluble protein within the mitochondrial matrix and plays a role in the attachment of the FAD cofactor to SDH (5).
Hereditary paragangliomas in the Netherlands are frequently caused by mutations in the SDHD gene, but mutations in SDHAF2, SDHB, and SDHC are also found (4, 6-9). Founder mutations in SDHD including the c.274G>T, p.Asp92Tyr mutation and the c.416T>C, p.Leu139Pro mutation play a major role in the prevalence of hereditary paraganglioma in the Netherlands (8). More recently, two founder mutations in SDHB were identified in Dutch paraganglioma and pheochromocytoma families (6, 10). The c.232G>A, p.Gly78Arg mutation is the only SDHAF2 mutation found in Dutch paraganglioma patients, and all patients share a common ancestor (4). To date, no *SDHC*-linked paraganglioma families have been described in the Netherlands.
In this study, we describe the frequency of mutations in *SDHB*, *SDHC*, *SDHD* or *SDHAF2* in 1045 paraganglioma and pheochromocytoma patients and their relatives. The results were obtained from the Leiden University Medical Center (LUMC), a dedicated referral center for paragangliomas and the primary referral laboratory for SDH mutation analysis in the Netherlands. As almost all Dutch paraganglioma patient samples are analysed here, the results represent the actual prevalence of mutations in genes encoding subunits of the SDH complex in the Netherlands.

Materials and methods

Patients
Peripheral blood leukocyte DNA from paraganglioma and pheochromocytoma patients and their relatives were collected from 1990-2009, at the Department of Human Genetics and the Laboratory for DNA Diagnostics of the Leiden University Medical Center (LUMC), the primary national referral center for SDH mutation scanning. The majority of DNA samples from patients and their relatives were sent for genetic analysis only, with only summary clinical data. Reason for referral was diagnosis of ‘paraganglioma’, ‘pheochromocytoma’, ‘chemodectoma’ or ‘glomus tumor’, in all cases. As the nomenclature of paragangliomas is not unequivocal and has changed over time, exact data regarding tumor location (*i.e.*, head- and neck region, adrenal medulla or extra-adrenal) are therefore unavailable for many patients, and are not further discussed. Cases were considered to be familial if two or more

affected individuals were identified within the same kindred. An index case is defined as the initial patient that presented with paraganglioma.

Mutation and deletion screening

The SDHB, SDHC, and SDHD genes were scanned for the presence of mutations from 2000-2009. All exonic regions of these genes were tested by direct sequencing using the Sanger method on an ABI 3177 Genetic Analyzer, starting with the exons containing the known Dutch founder mutations in SDHD followed by exons that had previously been found to contain pathogenic mutations in SDHB, SDHC, and SDHD (in that order) in the Dutch population. If this analysis was negative, scanning was completed by analyzing the remaining exons of these genes. After the identification of large founder mutation-related families in the Netherlands, the current strategy is to scan the SDHB, SDHC, and SDHD genes as indicated by the clinical phenotype or as requested by the submitting clinician. In 2007, mutation-negative cases were retrospectively analysed with MLPA for the presence of large deletions in SDHB, SDHC, and SDHD. MLPA has been carried out on all mutation-negative cases since then, using the P226 MLPA kit (MRC Holland, Amsterdam) containing probes for all exons and the promoter of each of these genes (27 different probes), according to the manufacturer's protocol. In cases with a negative SDHB, SDHC and SDHD mutation analysis, SDHAF2 was tested, as recently described (5). Informed consent was obtained for DNA testing according to protocols approved by LUMC Ethics Review Board.

Results

A total of 1045 samples from paraganglioma and/or pheochromocytoma patients and their relatives were analyzed for mutations in SDH-related genes. Mutations in SDHB, SDHC, SDHD or SDHAF2 were found in 690 cases, 239 of whom were index cases (Table 1). No mutations in SDH genes were found in 101 index cases and 254 family members of SDH mutation carriers (Table 1). The majority of SDH mutation carriers in the Netherlands carry a mutation in SDHD (87.1%), followed by mutations in SDHAF2 (6.7%), SDHB (5.9%) and SDHC (0.3%). By far the most prevalent mutation is the c.274G>T, p.Asp92Tyr mutation in SDHD, accounting for 69.1% of all SDH mutation carriers (Fig. 1). In all, 88.8% of the SDH mutation carriers in the Netherlands carried one of six Dutch founder mutations in the SDHD, SDHB or SDHAF2 genes (Table 2 and Fig. 1). A total of 340/1045 cases tested for SDH mutations were index cases. Mutations in SDHAF2, SDHB, SDHC or SDHD were identified in 239/340 (70.2%) index cases, most frequently a mutation in SDHD (62.1%) (Table 1). The most prevalent mutation among index cases was also the c.274G>T, p.Asp92Tyr mutation in SDHD, accounting for 157/239 index mutation carriers (65.6%).

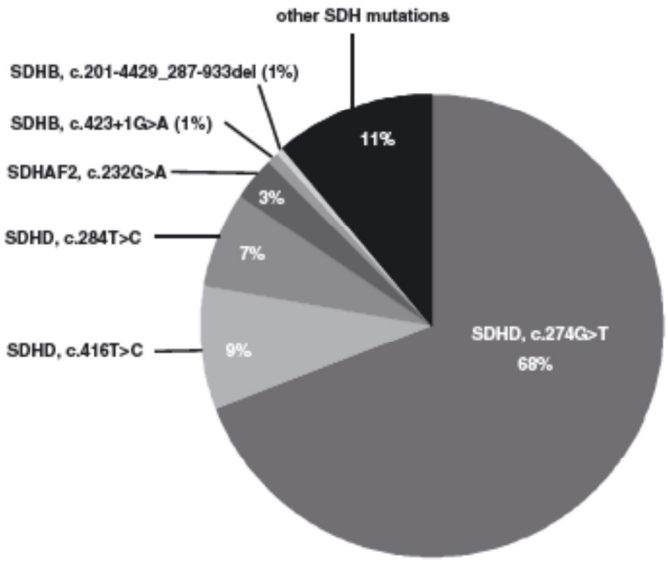
Table 1: Mutations in SDHAF2, SDHB, SDHC, and SDHD.

Gene	Exon	DNA mutation	Protein mutation	Cases (n)	Index cases(n)
SDHAF2	4	c.232G>A	p.Gly78Arg	46	4
SDHB	2	c.136C>T	p.Arg46X	3	2
	2	c.141G>A	p.Trp47X	1	1
	3	c.201-4429_287-933del	deletion exon 3	5	5
	3	c.268C>T	p.Arg90X	5	1
	4	c.343C>T	p.Arg115X	2	1
	4	c.423+1G>A	Splicesite	22	9
	6	c.574T>C	p.Cys192Arg	1	1
	6	c.590C>G	p.Pro197Arg	1	1
	7	c.653G>C	p.Trp218Ser	1	1
SDHC	4	c.214C>T	p.Arg72Cys	1	1
	5 & 6	c.242-241_510+1978del	deletion exon 5 & 6	1	1
SDHD	1 & 2	c.1-8828_169+442 del	deletion exon 1 & 2	1	1
	2	c.54_55dupC	p.Leu19ProfsX50	8	1
	2	c.64C>T	p.Arg22X	3	1
	2	c.112C>T	p.Arg38X	8	1
	2	c.120_121insC	p.Glu42ArgfsX27	6	3
	2	c.169_169+9 del TGTATGTTCT	unknown	4	1
	3	c.181delG	p.Ala61LeufsX25	2	1
	3	c.208A>G	p.Arg70Gly	1	1
	3	c.209G>T	p.Arg70Met	1	1
	3	c.242C>T	p.Pro81Leu	16	8
	3	c.274G>T	p.Asp92Tyr	477	157
	3	c.279T>G	p.Tyr93X	7	1
	3	c.284T>C	p.Leu95Pro	4	2
	3	c.287dupC	p.Ala97fs	1	1
	4	c.337_340 del GACT	p.Asp113MetfsX21	3	1
	4	c.416T>C	p.Leu139Pro	59	30

Table 2: Dutch founder mutations of the *SDHAF2*, *SDHB* and *SDHD* genes.

Gene	Mutation	Protein	References
SDHAF2	c.232G>A	p.Gly78Arg	Hao 2009 (5), Bayley 2010 (4)
SDHB	c.423+1G>A	splice site, intron 4	Hes 2010 (10)
	c.201-4429_287-933del	deletion exon 3	Bayley 2009 (6)
SDHD	c.274G>T	p.Asp92Tyr	Baysal 2000 (2) Taschner 2001 (8)
	c.416T>C	p.Leu139Pro	Dannenberg 2002 (24), Taschner 2001 (8)
	c.284T>C	p.Leu95Pro	Cremers 2002 (7), Dannenberg 2002 (24), Taschner 2001 (8)

Figure 1: Relative frequencies of Dutch SDH founder mutation carriers. The graph includes a total of 613 carriers of a founder mutation in a SDH related gene.



Discussion

The majority of SDH mutation carriers in the Netherlands harbour the c.274G>T, p.Asp92Tyr mutation in SDHD. Several very large families residing in the western part of the Netherlands are known to carry this mutation, all linked by a strong founder effect (11). The second most widespread SDH mutation in the Netherlands is the c.416T>C, p.Leu139Pro founder mutation in SDHD, but this mutation accounts for hardly more than 10% of the number of p.Asp92Tyr mutation carriers, emphasizing the dominant role of the former mutation. Compared to the high prevalence of SDHD mutations, SDHB mutations are far less common (87.1% vs. 5.9%), but the majority of SDHB mutation carriers also harbour known founder mutations, specifically the intron 4 splicesite mutation, c.423+1G>A or the exon 3 deletion, c.201-4429_287-933del (6, 9) (Tables 1 and 2).
The difference in prevalence between SDHB and SDHD mutation carriers may in part be attributable to the lower penetrance of SDHB mutations (10, 12-14). Despite their common forebears, most patients with a Dutch founder mutation in SDHB present without a family

history of paraganglioma, suggesting that many more SDHB mutation carriers await discovery (12-16).

We noted a remarkable 14-fold difference in the number of SDHD and SDHB mutation carriers, and even taking only index cases into account, SDHD mutation carriers still predominate with a ratio of around 10:1 (Table 1). None of the international studies that have reported variation in relative frequencies of SDHB and SDHD mutations in head and neck paraganglioma cases have described such a large difference. An recent large Italian study identified a 2 fold higher prevalence of SDHD mutations (17), while a broader European study showed an approximate 1:1 distribution of SDHB and SDHD mutation carriers (13). Other studies have shown a 2.7- 4.5-fold higher frequency of SDHB mutation carriers (12, 14). In general, SDHB mutations are more common than SDHD mutations, indicating that SDHB mutation carriers in the Netherlands only appear to be scarce due to the higher prevalence of SDHD founder mutations.

Haplotype studies of the most prevalent founder mutations have shown unequivocally that mutation carriers share a common haplotype surrounding the mutations, and therefore share a common ancestor. The Dutch SDHD mutation, p.Asp92Tyr, is estimated to be 200-960 years old based on coalescence time calculations, and all known Dutch carriers of the SDHAF2 mutation, p.Gly78Arg, share a common haplotype and have also been linked to a common ancestor (11, 18).

In addition to mutations of SDHB and SDHD, we identified 46 carriers of the c.232G>A, p.Gly78Arg mutation in SDHAF2. Four large SDHAF2-linked paraganglioma families from the South East Netherlands are now known to share a common ancestor, a male, born in 1771 and who married three times (19).These families have remained largely in the same area and the p.Gly78Arg mutation is a founder mutation in the South East Netherlands, accounting for a significant proportion of the paraganglioma cases seen in the region.

Paraganglioma syndrome due to mutations in SDHC is extremely rare in the Netherlands. We have identified only two SDHC mutations, c.242-241_510+1978del and c.214C>T, in two patients (0.3%). Like SDHB, SDHC mutations may have been underreported because of the often sporadic-like presentation of SDHC-linked paraganglioma syndrome (3, 20).

The remarkable prevalence of Dutch SDH founder mutations is most probably due to the unusual social and demographic history of the Netherlands. Until only a generation ago, Dutch society was highly segregated, primarily on the basis of religious differences. This segregation affected social, political and cultural life, and was further aided by socio-economic, geographic, and linguistic factors. These factors limited intermarriage until well into the twentieth century, and the creation of genetically isolated populations, facilitating the proliferation of Dutch founder mutations, both in SDHD and other disease genes (21). The p.Asp92Tyr founder mutation in SDHD shows a strong geographic focus even today (8, 11).

Mutations of SDHB, SDHC, SDHD and SDHAF2 each result in distinct hereditary paraganglioma syndromes, with differing modes of inheritance, penetrance, risk of pheochromocytoma, and risk of malignant paraganglioma, meaning that prior identification of the affected gene is essential to provision of effective genetic counselling to the individual patient (13, 14, 20). Several algorithms prioritizing gene-specific mutation

testing in paraganglioma patients have been proposed, based on phenotypic characteristics, and with the dual objectives of minimizing mutation screening and cost reduction (22, 23). Although these algorithms represent a useful starting point for genetic analysis, it is doubtful whether the effectiveness and outcome of such algorithms are universally applicable, as the a priori chance of finding a mutation in a specific gene differs from country to country. Recognition of regional differences in the prevalence of mutations will allow the tailoring of genetic screening on the basis of local knowledge.

The present study shows that the majority of mutations in SDH subunits or co-factors in the Netherlands involve SDHD, followed by SDHAF2, SDHB and SDHC, and the majority of mutation carriers harbour the Dutch SDHD founder mutation, p.Asp92Tyr. This finding is in strong contrast with the extensive genetic heterogeneity found elsewhere and underlines the importance of regional differences in the mutation spectrum of genes associated with hereditary paraganglioma syndrome.

References

1 Astuti D, Latif F, Dallol A, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. American Journal of Human Genetics 2001: 69: 49-54.

2 Baysal BE, Ferrell RE, Willett-Brozick JE, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 2000: 287: 848-51.

3 Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nature Genetics 2000: 26: 268-70.

4 Bayley JP, Kunst HP, Cascon A, et al. SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. Lancet Oncol 2010: 11: 366-72.

5 Hao HX, Khalimonchuk O, Schraders M, et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 2009: 325: 1139-42.

6 Bayley JP, Grimbergen AE, van Bunderen PA, et al. The first Dutch SDHB founder deletion in paraganglioma-pheochromocytoma patients. BMC Med Genet 2009: 10: 34.

7 Cremers CW, De Monnink JP, Arts N, Joosten FB, Kremer H, Hoefsloot L. Clinical report on the L95P mutation in a Dutch family with paraganglioma. Otol Neurotol 2002: 23: 755-9.

8 Taschner PEM, Jansen JC, Baysal BE, et al. Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes & Cancer 2001: 31: 274-81.

9 Bayley JP, van M, I, Weiss MM, et al. Mutation analysis of SDHB and SDHC: novel germline mutations in sporadic head and neck paraganglioma and familial paraganglioma and/or pheochromocytoma. BMC Med Genet 2006: 7: 1.

10 Hes FJ, Weiss MM, Woortman SA, et al. Low penetrance of a SDHB mutation in a large Dutch paraganglioma family. BMC Med Genet 2010: 11: 92.

11 van Schothorst EM, Jansen JC, Grooters E, et al. Founder effect at PGL1 in hereditary head and neck paraganglioma families from the Netherlands. Am J Hum Genet 1998: 63: 468-73.

12 Benn DE, Gimenez-Roqueplo AP, Reilly JR, et al. Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. J Clin Endocrinol Metab 2006: 91: 827-36.

13 Neumann HP, Pawlu C, Peczkowska M, et al. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 2004: 292: 943-51.

14 Ricketts CJ, Forman JR, Rattenberry E, et al. Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. Hum Mutat 2010: 31: 41-51.

15 Hensen EF, Jansen JC, Siemers MD, et al. The Dutch founder mutation SDHD.D92Y shows a reduced penetrance for the development of paragangliomas in a large multigenerational family. Eur J Hum Genet 2010: 18: 62-6.

16 Solis DC, Burnichon N, Timmers HJ, et al. Penetrance and clinical consequences of a gross SDHB deletion in a large family. Clin Genet 2009: 75: 354-63.

17 Mannelli M, Castellano M, Schiavi F, et al. Clinically guided genetic screening in a large cohort of italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas. J Clin Endocrinol Metab 2009: 94: 1541-7.

18 van Baars F, van den Broek P, Cremers C, Veldman J. Familial Non-Chromaffinic Paragangliomas (Glomus Tumors) - Clinical Aspects. Laryngoscope 1981: 91: 988-96.

19 van Baars F, Cremers C, van den Broek P, Geerts S, Veldman J. Genetic-Aspects of Non-Chromaffin Paraganglioma. Human Genetics 1982: 60: 305-9.

20 Schiavi F, Boedeker CC, Bausch B, et al. Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. Jama-Journal of the American Medical Association 2005: 294: 2057-63.

21 Zeegers MP, van PF, Vlietinck R, Spruijt L, Ostrer H. Founder mutations among the Dutch. Eur J Hum Genet 2004: 12: 591-600.

22 Erlic Z, Rybicki L, Peczkowska M, et al. Clinical predictors and algorithm for the genetic diagnosis of pheochromocytoma patients. Clin Cancer Res 2009: 15: 6378-85.

23 Cascon A, Lopez-Jimenez E, Landa I, et al. Rationalization of genetic testing in patients with apparently sporadic pheochromocytoma/paraganglioma. Horm Metab Res 2009: 41: 672-5.
24 Dannenberg H, Dinjens WN, Abbou M, et al. Frequent germ-line succinate dehydrogenase subunit D gene mutations in patients with apparently sporadic parasympathetic paraganglioma. Clin Cancer Res 2002: 8: 2061-6.

Chapter 3

Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas

N. van Duinen[1], D. Steenvoorden[1], I.P. Kema[2], J.C. Jansen[3], A.H.J.T. Vriends[4], J.P. Bayley[4] J.W.A. Smit[1], J.A. Romijn[1], E.P.M. Corssmit[1]

[1]Departments of Endocrinology and Metabolic Diseases, [3]Otorhinolaryngology, [4]Center of Human and Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands
[2]Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, the Netherlands

Journal of Clinical Endocrinology and Metabolism 2010 Jan; (95 (1):209-214

Abstract

Context*:* Patients with head-and-neck paragangliomas (HNPGL) are regularly screened for catecholamines excess. The clinical relevance of increased urinary secretion of 3-methoxytyramine (3MT) is unclear in HNPGL.

Objective: To assess the prevalence and the clinical, biochemical and radiological presentation of patients with HNPGL with increased urinary excretion of 3MT.

Patients and Methods: 136 consecutive patients with HNPGL were included and screened for catecholamine excess by measurement of 24-hr urinary excretion of (nor)metanephrine, (nor)epinephrine, VMA, dopamine and 3MT. In patients with catecholamine excess, abdominal/intrathoracic paragangliomas were excluded by ^{123}I-MIBG scintigraphy, MRI and/or CT.

Results: Urinary 3MT excretion was increased in 31 of the 136 patients (23 %). In 18 of these 31 patients this was the only sign of biochemical activity of HNPGL. Dopamine excretion was higher in subjects with increased 3-MT excretion (1.62±0.1 µmol/24h vs. 2.5±0.3 µmol/24h, $p<0.01$). Of the 136 HNPGL patients, 21 (15%) had excessive excretion of at least 1 catecholamine and/or their metabolites when 3MT excretion was not taken into account. With the inclusion of patients with excessive 3MT excretion, 39 (29%) had excessive catecholamine excretion. Patients with 3MT excess had significantly more complaints of palpitations ($p<0.01$), diaphoresis ($p=0.03$), collapse ($p<0.05$) and a higher pulse rate ($p<0.01$). Increased excretion of 3MT was not associated with particular types of HNPGL or genotypes.

Conclusions: A substantial number of HNPGL patients have biochemically active tumors, reflected in increased excretion of 3MT, associated with increased dopamine excretion. Some patients only display increased excretion of 3 MT, but not of other catecholamines or their metabolites.

Introduction

Head-and-neck paragangliomas (HNPGL) are rare neuroendocrine tumors derived from parasympathetic ganglia, whereas abdominal and thoracic paragangliomas are derived from sympathetic ganglia (1). The majority of sympathetic paragangliomas produce catecholamines, especially if they are located in the adrenals (pheochromocytomas) (2). Although HNPGLs have the potential to produce and secrete catecholamines(3), only a minority of these patients (4%) actually have biochemical evidence of catecholamine excess (3;4).

HNPGL can be positive for tyrosine hydroxylase, the enzyme which converts tyrosine to L-dihydroxyphenylalanine (L-dopa), the precursor of dopamine. This observation suggests that even biochemically inactive paragangliomas may have the enzymatic ability to synthesize catecholamines (5). Interestingly, dopamine functions as a neurotransmitter in HNPGLs (6-8). However, dopamine production or dopamine co-secretion has been reported mainly in metastatic extra-adrenal paragangliomas, which has been interpreted to be a reflection of dedifferentiation and to be predictive of malignant potential in preoperative patients with apparently non-metastatic paragangliomas (9-11). However, these data were predominantly reported in tertiary referral centers for malignant paragangliomas. Therefore, it is unclear to which extent dopamine production is present in HNPGL in general. Dopamine is converted to 3-methoxytyramine (3MT) by the enzyme catechol-O-methyltransferase. Eisenhofer *et al.* showed that plasma free 3MT may be useful for identification of paragangliomas that produce predominantly dopamine, whereas urinary excretion of dopamine is a relative insensitive and nonspecific marker for dopamine secreting tumors (9). Urinary excretion rates of 3MT have not been assessed in paraganglioma patients.

Therefore, the aim of the present study was to assess the prevalence of increased urinary 3MT excretion in HNPGL patients and to assess the clinical, biochemical and radiological characteristics of HNPGL patients with increased 3MT excretion by comparing patients with and without increased urinary excretion of 3MT.

Patients and Methods

We evaluated in a cross sectional study the clinical, biochemical and radiological data of all consecutive patients with HNPGL who were followed at the outpatient clinic of the Leiden University Medical Center, a tertiary referral center for patients with paragangliomas. Biochemical screening included the measurement of catecholamine excretion in 24 hour urinary samples: (nor)metanephrines, (nor)epinephrine, dopamine and 3-methoxytyramine.

All patients were investigated at the outpatient clinic according to structured clinical protocols, which were standard care. These included questions focused at tumor- and catecholamine related signs and symptoms, measurement of blood-pressure in the supine position, and after 5 minutes of upright position, in order to screen for orthostatic hypotension. Repetitive head-and-neck MRI was performed with intervals of at least 2 years. Urine was collected during 24 hours in duplicate under strict dietary regulations

(patients abstained from pineapple, avocado, bananas, kiwi, nuts, plums, coffee, tea and other caffeine containing beverages) and after withdrawal of medication for several weeks or after changing antihypertensive medication to doxazosine. In case of excessive catecholamine excretion (*i.e.*, any value above the upper reference limit in two urine samples), radiological assessment by MIBG-scans and MRI and/or CT scans of thorax and abdomen were performed to identify the source of excessive catecholamine production. All patients with documented paragangliomas in the abdomen or thorax were excluded in the present study, because the interpretation of the biochemical results in relation to HNPGL could be confounded by the presence of these other paragangliomas.

We performed screening for SDH mutations in those HNPGL patients, who agreed to genetic testing. Hereditary disease was diagnosed on the basis of the presence of mutations of SDHB, SDHC, or SDHD in the patient and/or a family member.

We screened 156 consecutive HNPGL patients for catecholamine excess, including dopamine and 3-methoxytyramine excretion. Nineteen of these 156 patients were excluded from the current study because HNPGL had previously been resected and, therefore, these patients were without HNPGL at the moment of the current study. One patient with elevated 3-methoxytyramine excretion was excluded from the current analyses, because no additional radiological assessment was performed to exclude the presence of other paragangliomas not related to the head and neck regions. Therefore, the study group comprised of 136 patients with HNPGL.

The study was an evaluation of routine patient care. According to the requirements of Dutch law, it was not necessary to obtain permission from the institutional ethical commission. Prior to germ line mutation testing, informed consent was obtained from each patient.

Assays

Epinephrine, norepinephrine and dopamine excretion in 24 h urine collections were quantified by reversed high pressure liquid chromatography (HPLC) equipped with an electrochemical detector. Inter- and intra-assay coefficients of variations (CVs) for epinephrine were 4.3-9.0% ranging from high to low levels. For norepinephrine these data are 2.7-3.6% and for dopamine 3.1-4.8%. Vanillylic mandelic acid (VMA) excretion in urine was measured using HPLC with fluorometric detection with inter- and intra-assay CVs of 2.4-9.1%. (Nor) metanephrine and 3MT were determined by stable isotope mass fragmentography. The CVs f the 3-O-methylated catecholamine metabolites (nor)metanephrine and 3MT ranged from 1.7 to 4.2% (12).

Reference ranges were obtained in healthy volunteers. These values were for norepinephrine 0.06-3.40 µmol/24h, epinephrine <0.16 µmol/24h, dopamine 0.46-3.40 µmol/24h, VMA <30 µmol/24h, metanephrine 33-90 µmol/mol creatinine, normetanephrine 64-260 µmol/mol creatinine and 3MT 45-197 µmol/mol creatinine (13). SDH mutation analysis was performed by restriction digestion as described by Taschner *et al* (14;15).

Data analysis

SPSS for windows version 16.0 (SPSS inc., Chicago, IL) was used for data analysis. Results are expressed as means ± standard error (SE), unless specified otherwise. Independent sample t-tests and chi-square tests were used to compare patients with and without increased urinary excretion of 3MT. The average value of catecholamine excretion rates of two urine samples was used for calculation of p values. A p-value <0.05 was considered to represent a significant difference.

Results

Clinical and biochemical data

All 136 patients had HNPGL, because the presence of HNPGL was an inclusion criterion (Table 1). Urinary excretion of 3MT excretion was increased in 31 patients (23 %) (Table 2). In these patients the mean excretion of dopamine was also significantly increased ($p<0.01$) compared to the subjects without increased 3MT excretion.

In the 31 patients with increased 3MT excretion, 13 patients (42%) also had increased 24-hour urinary excretion of other catecholamines. One patient also had elevated urinary excretion of norepinephrine, 3 patients of VMA, 4 patients of metanephrine, 6 patients of normetanephrine and 5 patients of dopamine (Table 2).

Eight of the 105 patients with normal 3MT excretion (8%) had increased urinary excretion of other catecholamines. Four patients had elevated excretion of VMA, 3 of metanephrine, 1 of normetanephrine and 1 of dopamine.

When the 3MT results were excluded from the analysis, 21 of the 136 patients (15%) had excessive secretion of 1 or more catecholamines and/or their metabolites. When increased excretion of 3MT was taken into account, 39 patients had excessive excretion of catecholamines (29%). In other words, these data indicate that 18 patients had increased excretion of 3-methoxytyramine without excessive excretion of other catecholamines.

Clinical characteristics of patients with and without increased 3-methoxytyramine excretion (Table 3)

Patients with increased excretion of 3MT had more signs and symptoms (n=24, 80%) compared to the patients with normal 3-methoxytyramine excretion (n=59, 56%, $p=0.01$), including more complaints of palpitations ($p<0.01$), diaphoresis ($p<0.03$), collapse ($p<0.05$) and a higher pulse rate ($p=0.01$). In both patient groups hypertension was the most common clinical symptom.

Table 1: Clinical characteristics of 137 HNPGL patients with and without excessive urinary 3-methoxytyramine excretion

	Urinary 3-methoxytyramine excretion		
	Normal	Elevated	
Patient characteristics	N=105	N=31	p-value
Age (yrs)	49.3 ± 1.3	49.9 ± 2.2	0.82
Gender N (%)			0.07
Men	60 (57%)	12 (39%)	
Women	45 (43%)	19 (61%)	
BMI (kg/m^2)	24.6 ± 0.37	26.1 ± 1.4	0.31
Systolic BP (mm Hg)	133.6 ± 1.9	139.1 ± 2.9	0.16
Diastolic BP (mmHg)	80.2 ± 1.0	83.3 ± 2.0	0.16
Heart Ratio (beats/min)	71.3 ± 0.9	77.6 ± 1.9	<0.01
Type of glomustumor			
Caroticum	77 (74%)	21 (68%)	0.54
Jugulare	13 (12%)	3 (10%)	1.00
Vagale	43 (41%)	18 (58%)	0.09
Jugulotympanicum	14 (13%)	7 (23%)	0.26
Tympanicum	8 (8%)	4 (13%)	0.47
Number of HNPGL			
1	49 (47%)	9 (29%)	0.08
2	30 (29%)	12 (39%)	0.28
3	16 (15%)	6 (19%)	0.58
>3	10 (10%)	4 (13%)	0.74
	77 (73%)	26 (84%)	
No of patients with genetic analysis	61 (58%)	16 (52%)	
SDHD	4 (4%)	3 (10%)	
SDHD* fam	65 (62%)	19 (61%)	
SDHD total	5 (5%)	3 (10%)	0.95
SDHB	0	1 (3%)	
SDHB** fam	5 (5%)	4 (13%)	
SDHB total	1 (1%)	0	0.21
SDHC			
No mutation found	6 (6%)	3 (10%)	

* SDHD mutation characterized in family member; ** SDHB mutation characterized in family member.

Table 2: Urinary catecholamine excretion in 137 HNPGL patients, with and without increased 3-methoxytyramine excretion

	Urinary 3-methoxytyramine excretion				
	normal	elevated	normal	elevated	
catecholamines	N tested pos	N tested pos	Mean ± SE	Mean ± SE	p-value
Epinephrine (µmol/24 h)	0	0	0.02 ± 0.0	0.02 ± 0.0	0.45
Norepinephrine (µmol/24 h)	0	1 (3 %)	0.34 ± 0.1	0.55 ± 0.2	0.21
Dopamine (µmol/24 h)	1 (1 %)	5 (16 %)	1.62 ± 0.1	2.5 ± 0.3	<0.01
VMA (µmol/24 h)	4 (4 %)	3 (10 %)	21.7 ± 0.6	24.4 ± 2.2	0.25
Metanephrine (µmol/molkre)	3 (3 %)	4 (13 %)	52.1 ± 2.0	52.1 ± 2.0	0.04
Normetanephrine (µmol/molkre)	1 (1 %)	6 (19 %)	140 ± 5.0	300 ± 72	0.03
3M-tyramine (µmol/molkre)	0	31 (100 %)	107 ± 6.0	971 ± 219	<0.001

A patient is considered to be positive for the urinary excretion of a specific catecholamine or metabolite if the value is above the reference limit in two consecutive urine samples.

Radiological and MIBG assessment (table 1)

Radiological assessment of head and neck

Localization studies of the head and neck region were performed in all patients. Radiological evaluation of the head and neck region was performed by MRI in 95% of patients, and by CT-scanning in 6% of the patients. We refer to Table 1 for further details of the type and number of HNPGL.

There was no significant difference in the number and types of HNPGL between the two groups.

Most patients with HNPGL (n=78) had multiple HNPGL, which prohibited the identification of a culprit lesion of 3-methoxytyramine excretion. We identified 58 patients with only a single HNPGL, 49 patients with normal 24h urinary excretion rates of 3MT,and 9 patients with increased excretion rates of 3MT . Compared to patients with normal 24h urinary excretion rates of 3MT , the maximal tumor diameter tended to be higher in patients

with increased 24 h rates of 3MT excretion (2.8 ± 0.3 vs. 4.2 ± 0.7cm (mean diameter ± SE, p = 0.08).

Table 3: Signs and symptoms of 137 HNPGL patients, with and without increased 3-methoxytyramine excretion

	Urinary 3-methoxytyramine excretion		
	normal	elevated	
	n (%)	n (%)	p-value
Symptoms/ signs	59 (56%)	24 (80%)	0.01
Dizziness	6 (6%)	4 (13%)	0.23
Tinnitus	18 (17%)	4 (13%)	0.78
Hearing loss	23 (22%)	9 (30%)	0.47
Hoarseness	8 (8%)	5 (17%)	0.16
Difficulty swallowing	8 (8%)	3 (10%)	0.71
Palpitations	3 (3%)	6 (20%)	<0.01
Diaphoresis	4 (4%)	5 (17%)	0.03
Headache	4 (4%)	1 (3%)	1.00
Flushes	3 (3%)	0	1.00
Nausea	0	0	-
Vomiting	0	0	-
Pharyngeal fullness	3 (3%)	0	1.00
Cranial nerve palsy	3 (3%)	3 (10%)	0.12
Collapse	0	2 (7%)	<0.05
Hypertension (>140/90) mmHg)	25 (24%)	11 (39%)	0.13
Hypotension (<90/ 60 mmHg)	0	1 (4%)	0.22

Additional radiological analysis

In the 31 patients with increased 3MT excretion, an MIBG scan was performed in 27 patients (87%). In 11 of these 27 patients (41%), the MIBG scan showed increased uptake in the head and neck region, but not elsewhere in the body. In addition, in 13 of these 31 patients abdominal/ thoracic paragangliomas were excluded by MRI, in 11 patients abdominal paragangliomas were excluded by MRI**,** in 1 patient abdominal paragangliomas were excluded by CT, in 3 patients abdominal and thoracic paragangliomas were excluded by MRI, in 1 patient abdominal paragangliomas were excluded by MRI and thoracal paragangliomas were excluded by CT. In 2 patients paragangliomas were excluded only by MIBG-scintigraphy. In the 105 patients with normal excretion of 3-methoxytyramine, a MIBG scan was performed in 36 patients (34%). In 18 of these 36 patients (50%), the MIBG scan showed increased uptake in the head and neck region, but not elsewhere in the

body. In the 8 patients with increased urinary catecholamine excretion, abdominal and/or thoracic paragangliomas were excluded by MRI and MIBG scans.

Discussion

This study documents that 23 % of HNPGL patients have increased urinary excretion of 3MT. When all biochemical data were included, 29% of all HNGPL patients had increased excretion of catecholamines and/or their products. In some of the patients with increased excretion of 3MT, the excretion of other catecholamines or their metabolites was not increased, indicating that the biochemical activity of HNPGL was only reflected in increased excretion of the 3MT. The clinical manifestations in patients with increased 3MT excretion were different compared to patients with normal excretion of 3MT. Patients with increased excretion of 3MT suffered more frequently from palpitations, diaphoresis, collapse and had a higher pulse rate. Therefore, our study results indicate that the prevalence of biochemically active HNPGL is much higher than hitherto appreciated in studies which did not include urinary 3MT measurements (4).

Patients with HNPGL frequently have multifocal tumors, which was also apparent in the present study. Consequently, it is difficult to identify the culprit lesion of 3MT excretion. Nonetheless, it is evident from the present study that each type of head and neck paraganglioma can be associated with increased 3MT excretion.

Urinary dopamine is primarily derived from renal extraction and decarboxylation of circulating DOPA, the precursor of dopamine. In addition, HNPGL that produce dopamine as the main catecholamine, can be identified by 24 hour urinary excretion of 3MT, and this O-methylated metabolite of dopamine provides a better marker for these tumors than does urinary excretion of dopamine. Eisenhofer *et al.* reported in patients with adrenal and extra-adrenal paragangliomas that plasma methoxytyramine was a better marker than urinary excretion of dopamine (9). We found that only part of the HNPGL patients with increased urinary excretion of 3MT also had increased urinary dopamine excretion. Therefore, urinary 3MT excretion is more sensitive in discovering dopamine-producing HNPGL than urinary dopamine excretion. Additional studies comparing plasma dopamine and 3MT levels and 24 hour urinary excretion of dopamine and 3MT are needed to assess the diagnostic sensitivity and benefits of these measurements in patients with HNPGL.

Only one previous study evaluated catecholamine production by HNPGL, which reported a prevalence of 4% of biochemically active HNPGL (4). Catecholamine excess was defined in that study by “increased excretion of one or more catecholamines or their metabolites above the cut-off point in at least one 24 hour urinary sample”, whereas in our study a more stringent criterion of catecholamine excess in two 24 hour urinary samples was required. Moreover, in that study not every patient with HNPGL was screened for catecholamine excess. In addition, measurement of urinary excretion of VMA was not included in their biochemical screening. In our study, exclusion of the VMA results reduced the incidence of catecholamine excess to 12% of the 136 patients. Therefore, it is very likely that the prevalence of biochemically active HNPGL is much higher than hitherto appreciated. The biochemical proof of HNPGL associated catecholamine excess is normalization of

catecholamine excess after resection of HNPGL. However, since most of HNPGL grow very slowly (16), and operative resection is accompanied by considerable morbidity (17), for the majority of patients a wait-and-scan strategy is advised. Nonetheless, our study shows that the incidence of catecholamine secreting HNPGL is higher than currently appreciated.

Although dopamine secreting paragangliomas are often asymptomatic (9), excessive excretion of dopamine can cause complaints of nausea, vomiting, flushing and orthostatic hypotension (18;19). We observed that the clinical manifestations in patients with increased 3MT excretion were different compared to those in patients with normal excretion of 3MT. Moreover, there was no difference in the presence of hyper- or hypotension between patients with and without increased excretion of 3MT.

Dopamine production or co-secretion has been interpreted as a sign of dedifferentiation and to be predictive of malignant potential (9-11). Our cohort consists of patients with only benign HNPGL, either sporadic or as a part of a familial syndrome caused by SDHB or SDHD mutations. There were no differences in the prevalence of mutations in these two genes between HNPGL patients with and without increased 3MT excretion. In addition, our results show that increased excretion of 3MT, and the associated increase in dopamine excretion, is not associated with malignant paragangliomas.

In conclusion, a relatively large number of patients with HNPGL have increased excretion of 3MT. Urinary 3MT excretion is a more sensitive marker than urinary dopamine excretion to identify patients with biochemically active HNPGL.

References

1 DeLellis RA, Lloyd RV, Heitz PU, Eng C. 2004 Pathology and genetics: WHO classification of tumours of endocrine organs. Oxford, UK: Oxford university Press.

2 Pacak K, Keiser H, Eisenhofer G. 2005 Pheochromocytoma, In: Textbook of endocrinology, 5th ed. Philadelphia: Elsevier Saunders, Inc.; 2501-2534.

3 Zak FG, Lawson W. The paraganglionic chemoreceptor system. 1st ed. New York: Springer-Verlag New York Inc., 1982.

4 Erickson D, Kudva YC, Ebersold MJ, Thompson GB, Grant CS, van Heerden JA, Young WF, Jr. 2001 Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. J Clin Endocrinol Metab 86:5210-5216

5 Timmers HJ, Kozupa A, Eisenhofer G, Raygada M, Adams KT, Solis D, Lenders JW, Pacak K 2007 Clinical presentations, biochemical phenotypes, and genotype-phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. J Clin Endocrinol Metab 92:779-786

6 Jeffery J, Devendra D, Farrugia J, Gardner D, Murphy MJ, Williams R, Ayling RM, Wilkin TJ 2006 Increased urinary dopamine excretion in association with bilateral carotid body tumours-- clinical, biochemical and genetic findings. Ann Clin Biochem 43:156-160

7 Koch CA, Rodbard JS, Brouwers FM, Eisenhofer G, Pacak K 2003 Hypotension in a woman with a metastatic dopamine-secreting carotid body tumor. Endocr Pract 9:310-314

8 Minguez-Castellanos A, Escamilla-Sevilla F, Hotton GR, Toledo-Aral JJ, Ortega-Moreno A, Mendez-Ferrer S, Martin-Linares JM, Katati MJ, Mir P, Villadiego J, Meersmans M, Perez-Garcia M, Brooks DJ, Arjona V, Lopez-Barneo J 2007 Carotid body autotransplantation in Parkinson disease: a clinical and positron emission tomography study. J Neurol Neurosurg Psychiatry 78:825-831

9 Eisenhofer G, Goldstein DS, Sullivan P, Csako G, Brouwers FM, Lai EW, Adams KT, Pacak K 2005 Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. J Clin Endocrinol Metab 90:2068-2075

10 John H, Ziegler WH, Hauri D, Jaeger P 1999 Pheochromocytomas: can malignant potential be predicted? Urology 53:679-683

11 Proye C, Fossati P, Fontaine P, Lefebvre J, Decoulx M, Wemeau JL, Dewailly D, Rwamasirabo E, Cecat P 1986 Dopamine-secreting pheochromocytoma: an unrecognized entity? Classification of pheochromocytomas according to their type of secretion. Surgery 100:1154-1162

12 Kema IP, Meiborg G, Nagel GT, Stob GJ, Muskiet FA 1993 Isotope dilution ammonia chemical ionization mass fragmentographic analysis of urinary 3-O-methylated catecholamine metabolites. Rapid sample clean-up by derivatization and extraction of lyophilized samples. J Chromatogr 617:181-189

13 Willemsen JJ, Ross HA, Wolthers BG, Sweep CG, Kema IP 2001 Evaluation of specific high-performance liquid-chromatographic determinations of urinary metanephrine and normetanephrine by comparison with isotope dilution mass spectrometry. Ann Clin Biochem 38:722-730

14 Bayley JP, van M, I, Weiss MM, Jansen JC, Oomen PH, Menko FH, Pasini B, Ferrando B, Wong N, Alpert LC, Williams R, Blair E, Devilee P, Taschner PE 2006 Mutation analysis of SDHB and SDHC: novel germline mutations in sporadic head and neck paraganglioma and familial paraganglioma and/or pheochromocytoma. BMC Med Genet 7:1

15 Taschner PE, Jansen JC, Baysal BE, Bosch A, Rosenberg EH, Brocker-Vriends AH, van der Mey AG, van Ommen GJ, Cornelisse CJ, Devilee P 2001 Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes Cancer 31:274-281

16 Jansen JC, van den BR, Kuiper A, van der Mey AG, Zwinderman AH, Cornelisse CJ 2000 Estimation of growth rate in patients with head and neck paragangliomas influences the treatment proposal. Cancer 88:2811-2816

17 Bradshaw JW, Jansen JC 2005 Management of vagal paraganglioma: is operative resection really the best option? Surgery 137:225-228

18 Dubois LA, Gray DK 2005 Dopamine-secreting pheochromocytomas: in search of a syndrome. World J Surg 29:909-913

19 Januszewicz W, Wocial B, Januszewicz A, Gryglas P, Prejbisz A 2001 Dopamine and dopa urinary excretion in patients with pheochromocytoma--diagnostic implications. Blood Press 10:212-216

Chapter 4

Plasma levels of free metanephrines and 3-methoxytyramine indicate a higher number of biochemically active HNPGL than 24 h urinary excretion rates of catecholamines and metabolites.

N. van Duinen[1], E.P.M. Corssmit[1], W.H.A de Jong[2], D. Brookman[1], I.P. Kema[2], J.A. Romijn[1]

[1]Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, the Netherlands

[2]Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, the Netherlands

Submitted for publication

Abstract

Context: A substantial number of patients with head and neck paragangliomas (HNPGL) have biochemically active tumors, evidenced by increased urinary excretion of catecholamines and metabolites, including 3-methoxytyramine (3MT). It is unclear whether plasma levels of these parameters are more sensitive to detect biochemical activity in HNPGL patients than urinary excretion rates.

Objective: To compare plasma free levels versus urinary excretion rates of deconjugated 3MT and combined metanephrines in patients with HNPGL.

Patients and Methods: We included 124 consecutive patients with HNPGL for screening for catecholamine excess by measurement of 24-hr urinary excretion rates of deconjugated (nor)metanephrine, (nor)epinephrine, dopamine, vanillylmandelic acid, 3MT and plasma free levels of (nor)metanephrine and 3MT.

Results: Plasma free 3MT levels were increased in 35 of the 124 patients (28%), whereas 24-h urinary excretion of deconjugated 3MT was increased in 30 patients (24%) (p=0.13). Plasma free metanephrine (MN) levels were increased in 7 patients (6%), urinary deconjugated metanephrine levels in 6 (5%) (p=1.00). Plasma free normetanephrine (NMN) levels were increased in 7 patients (6%), and 5 patients had increased urinary excretion of deconjugated normetanephrine (4%) (p=0.69). Plasma free combined metanephrine levels (NMN, MN, 3MT) were increased in 41 patients (33%), whereas 24-h urinary excretion rates of deconjugated combined metanephrines was increased in 33 patients (27%, $p<0.05$).

Conclusions: The combined levels of free metanephrines and free 3MT in plasma indicate a higher number of biochemically active HNPGL than the 24 h urinary excretion rates of these markers**.**

Introduction

Head and neck paragangliomas (HNPGL) are rare neuroendocrine tumors derived from parasympathic ganglia (1). Some patients with HNPGL have biochemically active HNPGL, evidenced by increased urinary excretion rates of catecholamines and their metabolites (2-4). The majority of those patients have increased urinary excretion rates of 3-methoxytyramine (3MT), the 3-O-methylated metabolite of dopamine (3).
It is presently unknown whether plasma free concentrations of 3-O-methylated metabolites of catecholamines, including 3MT, are more sensitive parameters of biochemical activity of HNPGL than urinary excretion rates of catecholamines or deconjugated 3-O-methylated metabolites. For the diagnosis of pheochromocytoma, the measurement of plasma free metanephrine concentrations is the optimal biochemical test with the highest sensitivity and specificity (5-8). Therefore, the aim of the present study was to assess whether plasma levels of free metanephrines and 3MT are more sensitive parameters of biochemical activity of HNPGL than urinary excretion rates of free catecholamines and their deconjugated metabolites.

Patients and Methods

We performed a cross sectional study of 130 consecutive patients with HNPGL who were followed at the outpatient clinic of the Leiden University Medical Center, a tertiary referral center for patients with paragangliomas. For this purpose clinical, biochemical and radiological data of all consecutive patients with HNPGL were evaluated. All patients were investigated at the outpatient clinic according to structured standard clinical protocols. These included questions focused at tumor and catecholamine related signs and symptoms, measurement of blood-pressure in the supine position, and after 5 minutes of upright position, in order to screen for orthostatic hypotension. In all patients head-and-neck MRI were performed or had been performed within the previous 2 years.
Urine was collected during 24 hours in duplicate under strict dietary regulations (patients abstained from pineapple, avocado, bananas, kiwi, nuts, plums, coffee, tea and other caffeine containing beverages) and after withdrawal of medication for at least one week or after changing antihypertensive medication to doxazosine for several weeks. In order to ascertain adequacy of urinary collection, 24-hour urinary creatinine excretion rates were measured as well. Blood samples were drawn after the second day of urine collection in the postabsorptive state. Blood samples were drawn from an intravenous catheter inserted into a forearm vein after 30 minutes of rest in the supine position and collected in cold, glutathione containing vacutainers. All blood samples were centrifuged immediately at 3000 rpm for 10 min at 4 ºC. Plasma samples were stored at -80 ºC until analyses.
Biochemical screening included the measurement of urinary excretion rates of deconjugated (nor)metanephrine, free (nor)epinephrine, dopamine and deconjugated 3MT excretion in two 24-hour urinary samples, and the measurement of plasma free (nor)metanephrines and 3MT concentrations.

In case of excessive catecholamine or metabolite excretion (*i.e.*, any value above the upper reference limit in two urine samples or in the plasma sample), radiological assessment by MIBG-scans and MRI and/or CT scans of thorax and abdomen were performed to identify the source of excessive catecholamine production. All patients with documented paragangliomas in the abdomen or thorax were excluded in the present study, because the interpretation of the biochemical results in relation to HNPGL could be confounded by the presence of these other paragangliomas.

We performed screening for succinate dehydrogenase (SDH) mutations in those HNPGL patients, who agreed upon genetic testing. Hereditary disease was diagnosed if mutations in the SDHB, SDHC, or SDHD genes were documented in the HNPGL patient and/or a family member.

We screened 130 consecutive HNPGL patients for catecholamine excess. Four patients with catecholamine excess were excluded from the current analyses, because no additional radiological assessment was performed to exclude the presence of other paragangliomas not related to the head and neck regions. Two patients were excluded because they were diagnosed with a pheochromocytoma. Therefore, the study group comprised 124 patients with HNPGL.

The study was an evaluation of routine patient care. According to the requirements of Dutch law, it was not necessary to obtain permission from the institutional ethical commission. Prior to germ line mutation testing, informed consent was obtained from each patient.

Assays

Free Epinephrine, norepinephrine and dopamine excretion rates in 24 h urine collections were quantified by reversed phase high pressure liquid chromatography (HPLC) with electrochemical detection. Inter- and intra-assay coefficients of variations CVs for epinephrine were 4.3-9.0% ranging from low to high concentrations. For norepinephrine these data were 2.7-3.6% and for dopamine 3.1-4.8%. Vanillylmandelic acid (VMA) excretion in urine was measured using HPLC with fluorometric detection with inter- and intra-assay CVs of 2.4-9.1%. Urinary deconjugated (nor)metanephrine and 3MT were determined by isotope dilution gas chromatography with mass spectrometric detection. The CVs of the 3-O-methylated catecholamine metabolites ((nor)metanephrine and 3MT) ranged from 1.7-4.2% (9). Plasma free metanephrines were determined by automated in-line solid phase extraction and isotope dilution liquid chromatography with mass spectrometric detection (11). Reference ranges were obtained in healthy volunteers. These values were for urinary excretion: free norepinephrine 0.06-0.47 µmol/24h, epinephrine <0.16 µmol/24h, and dopamine 0.46-3.40 µmol/24h, VMA <30 µmol/24h (deconjugated), metanephrine (deconjugated) 33-99 µmol/mol creatinine, normetanephrine (deconjugated) 64-260 µmol/mol creatinine and 3MT (deconjugated) 45-197 µmol/mol creatinine (10). The reference intervals for plasma free metanephrines were determined using blood samples collected in the supine position from 115 volunteers (57 males, 58 females; age range, 36-81 years; median age, 55 years) (11) The reference ranges for plasma free metanephrines

were: metanephrine 0.07- 0.33 nmol/L, normetanephrine 0.23-1.07 nmol/L and 3MT <0.17 nmol/L. SDH mutation analysis was performed by restriction digestion as described by Taschner *et al* (12;13).

Data analysis

SPSS for windows version 16.0 (SPSS inc., Chicago, IL) was used for data analysis. Results are expressed as means ± standard error (SE), unless specified otherwise. Test sensitivity was calculated from the patients with a positive test result divided by the total number of patients tested. Test sensitivities were compared using the McNemar test. The average value of catecholamine and metabolite excretion rates of two urine samples was used for calculation of p values. A p-value <0.05 was considered to represent a significant difference.

Results

Clinical characteristics (Table 1)
The study group comprised 124 patients with HNPGL. Mean age of the patients was 49 years (range 13-77 years). Fifty-five patients (44%) had a single head and neck paraganglioma, whereas 69 patients (56%) had multiple head and neck paragangliomas. Paragangliomas occurred most frequently in the glomus caroticum (70%). Fifty-one patients (41%) had a glomus vagale tumor, 21 (17%) a glomus jugulotympanicum tumor, 13 (11%) a glomus jugulare tumor and 12 (10%) a glomus tympanicum tumor.
Genetic testing for SDHx mutations was performed in 111 of the 124 patients with HNPGL (90%). In 95 patients mutations were found (86 SDHD, 8 SDHB and 1 SDHC), whereas in 16 patients no mutation was found.

Biochemical characteristics (Table 2)
Forty-six patients (37%) had biochemically active HNPGL, evidenced by increased plasma concentrations of free (nor)metanephrine, 3MT and/or increased 24 hour urinary concentrations of free catecholamines, and deconjugated metabolites, including 3MT. There was no difference between the number of subjects with increased plasma free 3MT concentrations compared to the number of subjects with increased urinary excretion rates of deconjugated 3MT (n=35 vs. n=30, 28% vs. 24%, p=0.13). Urinary deconjugated metanephrine excretion rates were increased in 8% (10 of 124 patients), which in combination with the patients with increased urinary deconjugated 3MT concentration, increased the number of patients with biochemically active HNPGL to 27% (33 of 124 patients). Eleven patients (9%) had increased urinary excretion rates of VMA. Increased urinary excretion rates of free catecholamines (adrenaline, noradrenaline, or dopamine) were present in 10% of the patients (12 of 124 patients). Increased urinary excretion rates of combined metanephrines in combination with catecholamines were present in 28% of the patients (35 of 124 patients).

Plasma free (nor)metanephrine concentrations were increased in 11% of the patients (14 of 124 patients). The addition of plasma free 3MT levels to these measurements increased the portion of patients with evidence of biochemically active HNPGL to 33% (41 of 124 patients). The number of HNPGL patients with increased plasma free metanephrine levels was not significantly different from the number of patients with increased 24h urinary excretion rates of deconjugated metanephrines (11% vs. 8%, p=0.34). Increased plasma free metanephrine (MN, NMN) concentrations in combination with increased plasma free 3MT levels were present in significantly more HNPGL subjects than increased urinary excretion rates of combined metanephrines (MN, NMN) including 3MT (33% vs. 27%, p<0.05).
Nine patients tested negative with urinary screening of catecholamine excretion rates but positive with screening of plasma metanephrine levels. Of these patients 6 had increased plasma free 3MT levels, 2 had increased plasma free metanephrine levels and 1 had increased plasma free normetanephrine levels.

Catecholamine and metabolite excretion and tumor load
We identified 55 patients with only a single HNPGL with a mean tumor diameter of 3.3 cm (range 0.3-9.7 cm). In those patients there was a weak positive correlation between tumor diameters and plasma concentrations of free 3MT (r=0.30, p=0.04), and the combined plasma concentrations of free metanephrine, normetanephrine and 3MT (r=0.35, p=0.02). There were also weak correlations between tumor diameters and mean urinary excretion rates of deconjugated normetanephrine (r=0.40, p<0.01), VMA (r=0.32, p=0.03), dopamine (r=0.40, p<0.01), the combined excretion rates of deconjugated metanephrine and normetanephrine (r=0.40, p<0.01), the combined excretion rates of deconjugated (nor)metanephrine and 3MT (r=0.43, p<0.01) and the mean excretion rate of catecholamines (r=0.37, p=0.01). There was no correlation between the number of HNPGL and plasma free metanephrine levels, mean urinary excretion rates of deconjugated metanephrines and catecholamines.

Catecholamine and metabolite excretion, age, BMI and gender
Stepwise linear regression analysis was performed in a model including age, gender and BMI as independent variables and plasma free metanephrine, normetanephrine and 3MT levels as dependant variables. We identified age (β=0.35, p<0.001), BMI (β=-0.29, p<0.001) and gender (β=0.35, p<0.001) as independent predictors of plasma free metanephrine levels. The patients with increased plasma free metanephrine levels were significantly older compared to patients with normal levels (48.0±1.2 vs. 61.5±3.7 yrs, p<0.01). Men had significantly higher plasma free metanephrine levels compared to women (0.21±0.0 vs. 0.15±0.0, p<0.001).
For plasma levels of free normetanephrine there was no relation with age (β=0.09, p=0.35), BMI (β=0.11, p=0.26) and gender (β=0.09, p=0.35). There was a relation between age (β =0.09, p=0.38), BMI (β =0.02, p=0.41) or gender (β =0.08, p=0.41) with plasma free 3MT levels.

Table 1: Clinical characteristics of 124 patients with head and neck paragangliomas (HNPGL).

	All patients with HNPGL N= 124	Hereditary HNPGL N= 95	Sporadic HNPGL N= 16
Patient characteristics			
Age (yr)	49 ± 1	47 ± 1	58 ± 3
Gender N (%)			
Men	64 (52%)	51 (54%)	4 (25%)
Women	60 (48%)	44 (46%)	12 (75%)
Type of glomus tumor N (%)			
Caroticum	87 (70%)	73 (77%)	4 (25%)
Vagale	51 (41%)	42 (44%)	6 (38%)
Jugulare	13 (11%)	11 (12%)	1 (6%)
Jugulotympanicum	21 (17%)	13 (14%)	5 (31%)
Tympanicum	12 (10%)	6 (6%)	5 (31%)
No. of HNPGL N (%)			
1	55 (44%)	37 (39%)	12(75%)
>1	69 (56%)	58 (61%)	4 (25%)
No. of patients with genetic analysis			
SDHD	86 (69%)		
SDHB	8 (7%)		
SDHC	1 (1%)		
Sporadic	16 (13%)		

Data are shown as mean ± SEM, unless mentioned otherwise.

Discussion

The results of this study show that one third of HNPGL patients have biochemically active HNPGL. The combined plasma concentrations of free metanephrines and 3MT indicate a higher proportion of patients with biochemically active HNPGL than 24 h urinary excretion rates of deconjugated metanephrines and 3MT. In addition, the current data indicate that in HNPGL patients, plasma free 3MT concentrations and urinary excretion rates of deconjugated 3MT do not indentify significantly different numbers of subjects with biochemically active HNPGL.

HNPGL have the ability to produce and secrete catecholamines (2;3). Biochemically active paragangliomas are identified by the measurement of plasma (nor)metanephrine and 3MT

concentrations and 24h urinary excretion rates of free catecholamines and their deconjugated metabolites (6;14). The measurements of urinary excretion rates of catecholamine metabolites (*i.e.*, metanephrine and normetanephrine) and especially plasma levels of catecholamines and their metabolites are recommended for the biochemical screening of pheochromocytoma because of their
high diagnostic sensitivity and specificity (7). However, the majority of patients with biochemically active HNPGL secrete 3MT, which is a metabolite of dopamine. Therefore, the measurement of this metabolite should be included if biochemical activity of HNPGL in general is assessed. Three-methoxytyramine can be measured in urine and plasma by HPLC-tandem mass spectrometric detection (XLC-MS/MS) (11). The present study indicates that the assessment of plasma free 3MT levels does not add to the measurement of urinary excretion rates of deconjugated 3MT.

Table 2: Plasma metanephrine levels and mean urinary excretion rates of catecholamines, VMA and metanephrines in 124 HNPGL patients.

		Mean (range)	N tested positive
Plasma			
Normetanephrine	(nmol/L)	0.6 (0.16-6.64)	7 (6%)
Metanephrine	(nmol/L)	0.18 (0.04-0.48)	7 (6%)
3-methoxytyramine	(nmol/L)	0.46 (0.03-12.8)	35 (28%)
MN + NMN	(nmol/L)	0.78 (0.28-6.78)	14 (11%)
MN + NMN + 3MT	(nmol/L)	1.24 (0.32-13.9)	41 (33%)
Urine			
Normetanephrine	(µmol/mol creatinine)	176 (55-1720)	5 (4%)
Metanephrine	(µmol/mol creatinine)	55 (16-162)	6 (5%)
3-Methoxytyramine	(µmol/mol creatinine)	312 (43-6391)	30 (24%)
MN + NMN	(µmol/mol creatinine)	232 (95.5-1783)	10 (8%)
MN + NMN + 3MT	(µmol/mol creatinine)	544 (154-6598)	33 (27%)*
Adrenaline	(µmol/24 h)	0.02 (0.0-0.11)	0
Noradrenaline	(µmol/24 h)	0.40 (0.11-5.2)	6 (5%)
Dopamine	(µmol/24 h)	1.92 (0.77-6.43)	8 (7%)
Catecholamines	(µmol/24 h)	2.34 (0.91-9.78)	12 (10%)
VMA	(µmol/24 h)	22.5 (0-62)	11 (9%)
Catecholamines + combined MNs			35 (28%)

*indicates a significant difference in test sensitivity between urine and plasma.
VMA= Vanillylmandelic acid; combined MNs: MN + NMN + 3MT.

As patients with HNPGL have the ability to (co)secrete noradrenaline (15-17), this catecholamine and its metabolite normetanephrine should be measured as well. Combining the results of free metanephrines and 3MT in plasma resulted in a slightly, but significantly, higher number of patients with biochemically active HNPGL compared to the combined results of urinary excretion rates.

Consumption of catecholamine rich food products can result in substantial increases in urinary excretion rates of deconjugated normetanephrine and 3MT, and to a lesser extend in plasma free 3MT levels. Therefore, dietary restrictions are indicated prior to collection of blood for measurements of plasma free 3MT levels, and urinary excretion rates of deconjugated normetanephrine and 3MT (18). Although patients in our study collected urine during 48 hour under strict dietary regulations, we can not exclude potential confounding effects of the diet. In contrast, the measurements of plasma free metanephrine concentrations are not influenced by the confounding effects of dietary components, this contributes to the highest sensitivity of plasma levels compared to the measurement of urinary excretion rates of metanephrine (18;19). In accordance, plasma free metanephrines (MN, NMN, 3MT) levels were increased in a higher percentage of HNPGL patients compared to urinary excretion rates of metanephrines. Our findings are in agreement with the observations in patients with pheochromocytomas (6-8). Lenders *et al.* reported a test sensitivity of 97% of plasma free metanephrines versus only 60% of urinary combined metanephrines in patients with hereditary pheochromocytomas and 99% of plasma metanephrine levels versus 88% in urinary excretion rates in patients with sporadic pheochromocytomas.

In conclusion, one third of HNPGL patients have biochemically active HNPGL. The combined assessment of plasma concentrations of free metanephrines and 3MT detect a higher number of biochemically active HNPGL than the measurement of 24 h urinary excretion rates of combined metanephrines and 3MT. In addition, the current data indicate that in HNPGL patients urinary excretion rates of deconjugated 3MT and plasma free 3MT levels do not indentify significantly different numbers of subjects with biochemical active HNPGL.

References

1 DeLellis RA, Lloyd RV, Heitz PU, Eng C. World Health Organisation classification of tumors of endocrine organs. 2004. Oxford, UK: Oxford University Press.

2 Erickson D, Kudva YC, Ebersold MJ, Thompson GB, Grant CS, van Heerden JA, Young WF, Jr. 2001 Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. J Clin Endocrinol Metab 86:5210-5216

3 van Duinen N, Steenvoorden D, Kema IP, Jansen JC, Vriends AH, Bayley JP, Smit JW, Romijn JA, Corssmit EP 2010 Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. J Clin Endocrinol Metab 95:209-214

4 Zak FG, Lawson W. The paraganglionic chemoreceptor system. [1st ed. Springer-Verlag New York Inc.]. 1982.

5 Eisenhofer G, Lenders JW, Linehan WM, Walther MM, Goldstein DS, Keiser HR 1999 Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. N Engl J Med 340:1872-1879

6 Lenders JW, Keiser HR, Goldstein DS, Willemsen JJ, Friberg P, Jacobs MC, Kloppenborg PW, Thien T, Eisenhofer G 1995 Plasma metanephrines in the diagnosis of pheochromocytoma. Ann Intern Med 123:101-109

7 Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, Keiser HR, Goldstein DS, Eisenhofer G 2002 Biochemical diagnosis of pheochromocytoma: which test is best? JAMA 287:1427-1434

8 Sawka AM, Jaeschke R, Singh RJ, Young WF, Jr. 2003 A comparison of biochemical tests for pheochromocytoma: measurement of fractionated plasma metanephrines compared with the combination of 24-hour urinary metanephrines and catecholamines. J Clin Endocrinol Metab 88:553-558

9 Kema IP, Meiborg G, Nagel GT, Stob GJ, Muskiet FA 1993 Isotope dilution ammonia chemical ionization mass fragmentographic analysis of urinary 3-O-methylated catecholamine metabolites. Rapid sample clean-up by derivatization and extraction of lyophilized samples. J Chromatogr 617:181-189

10 Willemsen JJ, Ross HA, Wolthers BG, Sweep CG, Kema IP 2001 Evaluation of specific high-performance liquid-chromatographic determinations of urinary metanephrine and normetanephrine by comparison with isotope dilution mass spectrometry. Ann Clin Biochem 38:722-730

11 de Jong WH, Graham KS, van der Molen JC, Links TP, Morris MR, Ross HA, de Vries EG, Kema IP 2007 Plasma free metanephrine measurement using automated online solid-phase extraction HPLC tandem mass spectrometry. Clin Chem 53:1684-1693

12 Bayley JP, van M, I, Weiss MM, Jansen JC, Oomen PH, Menko FH, Pasini B, Ferrando B, Wong N, Alpert LC, Williams R, Blair E, Devilee P, Taschner PE 2006 Mutation analysis of SDHB and SDHC: novel germline mutations in sporadic head and neck paraganglioma and familial paraganglioma and/or pheochromocytoma. BMC Med Genet 7:1

13 Taschner PE, Jansen JC, Baysal BE, Bosch A, Rosenberg EH, Brocker-Vriends AH, van Der Mey AG, van Ommen GJ, Cornelisse CJ, Devilee P 2001 Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes Cancer 31:274-281

14 Eisenhofer G, Goldstein DS, Sullivan P, Csako G, Brouwers FM, Lai EW, Adams KT, Pacak K 2005 Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. J Clin Endocrinol Metab 90:2068-2075

15 Hall FT, Perez-Ordonez B, Mackenzie RG, Gilbert RW 2003 Does Catecholamine Secretion from Head and Neck Paragangliomas Respond to Radiotherapy? Case Report and Literature Review. Skull Base 13:229-234

16 Havekes B, van der Klaauw AA, Weiss MM, Jansen JC, van Der Mey AG, Vriends AH, Bonsing BA, Romijn JA, Corssmit EP 2009 Pheochromocytomas and extra-adrenal paragangliomas detected by screening in patients with SDHD-associated head-and-neck paragangliomas. Endocr Relat Cancer 16:527-536

17 van Houtum WH, Corssmit EP, Douwes Dekker PB, Jansen JC, van Der Mey AG, Brocker-Vriends AH, Taschner PE, Losekoot M, Frolich M, Stokkel MP, Cornelisse CJ, Romijn JA 2005 Increased prevalence

of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with SDHD-linked head and neck paragangliomas. Eur J Endocrinol 152:87-94

18 de Jong WH, Eisenhofer G, Post WJ, Muskiet FA, de Vries EG, Kema IP 2009 Dietary influences on plasma and urinary metanephrines: implications for diagnosis of catecholamine-producing tumors. J Clin Endocrinol Metab 94:2841-2849

19 Eisenhofer G, Coughtrie MW, Goldstein DS 1999 Dopamine sulphate: an enigma resolved. Clin Exp Pharmacol Physiol Suppl 26:S41-S53

Chapter 5

Plasma chromogranin A levels are increased in a small portion of patients with hereditary head and neck paragangliomas

N. van Duinen[1], I.P. Kema[2], J.A. Romijn[1], E.P.M. Corssmit[1]

[1]Departments of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, the Netherlands

[2]Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, the Netherlands

Clincal Endocrinology 2011 Feb;74 (2):160-165

Abstract

Context: The majority of patients with head and neck paragangliomas (HNPGL) have biochemically silent tumors. Chromogranin A (CgA) is a tumor marker for neuroendocrine tumors.

Objective: To assess the role of CgA as a tumor marker in patients with hereditary HNPGL.

Patients and Methods: We included 95 consecutive patients with hereditary HNPGL for screening of plasma CgA levels and catecholamine excess by measurement of 24-hour urinary excretion of (nor)metanephrine, (nor)epinephrine, VMA, dopamine and 3-methoxytyramine. In all patients with catecholamine excess, abdominal/intrathoracic paragangliomas were excluded by ^{123}I-MIBG scintigraphy, MRI and/or CT.

Results: Plasma CgA levels were increased in only 15 of 95 patients (16%). Thirty-three of the 95 patients (35%) had increased urinary excretion rates of catecholamines. Six of these 33 patients (18%) had increased plasma CgA levels. Nine of the 62 patients (15 %) with a biochemically silent tumor (*i.e.* no increased urinary excretion of catecholamines or their metabolites) had increased CgA levels.
Increased plasma CgA levels were positively correlated with urinary excretion rates of norepinephrine (r=0.68, p=0.005) and normetanephrine (r=0.68, p=0.005). There was a positive correlation between maximal HNPGL diameter and plasma CgA levels in the 57 patients with a single HNPGL (r=0.57, p=0.001).

Conclusions: Plasma CgA levels are increased in only a small portion of patients with hereditary HNPGL and have limited additional value to the combination of radiological and routine biochemical assessment of HNPGL patients. Increased plasma CgA levels are associated with increased noradrenergic activity and tumor size in patients with a single HNPGL.

Introduction

Head and neck paragangliomas (HNPGL) are rare neuroendocrine tumors derived from parasympathic ganglia (1). Paragangliomas can occur as a consequence of a mutation in genes of the succinate dehydrogenase (SDH) family (2-4). These SDH genes (SDHA, SDHB, SDHC and SDHD) encode the four subunits of complex II of the mitochondrial electron transport chain. SDH contributes to the energy metabolism as a component of the tricarboxylic acid cycle, converting succinate to fumarate, and by serving as a source of electrons for mitochondrial respiration, as complex II of the electron transport chain (5). Except for the SDHA gene, mutations of SDHB, SDHC and SDHD genes are associated with familial paraganglioma syndromes (2;6). In the Netherlands, a large proportion of hereditary paragangliomas are caused by mutations in the SDHD gene, but mutations in SDHB, SDHC and SDHAF2 are also found (5;7-10).
Although HNPGL have the ability to produce and secrete catecholamines (11;12), we recently demonstrated that only 29% of patients with HNPGL have evidence of increased urinary excretion of catecholamines and/or their metabolits (13). Consequenly, the majority of these patients have biochemical silent tumors and the clinical characteristics of these HNPGL can be evaluated only by imaging techniques.
Chromogranin A (CgA) is a secretory protein from neuroendocrine cells that mediates chromaffin granule biogenesis, necessary for catecholamine storage (14;15). CgA is secreted from neurosecretory vesicles, along with catecholamines (16). In accordance with these biological concepts, plasma CgA is a usefull tumor marker in patients with pheochromocytoma (17-23). Lloyd *et al.* demonstrated the presence of chromogranin in head and neck paraganglia, indicating the presence of secretory granules(24). At present the clinical relevance of the measurement of plasma CgA levels in patients with hereditary HNGPL is unclear. Therefore, the aim of the present study was to assess the prevalence of increased CgA levels in patients with hereditary HNPGL and to identify a possible role of CgA in patients with biochemically silent tumors.

Patients and Methods

We evaluated in a cross sectional study the clinical, biochemical and radiological data of 95 consecutive patients with hereditary HNPGL. These patients were selected from all HNPGL patients who were followed in the outpatient clinic of the Leiden University Medical Center, a tertiary referral center for patients with paragangliomas. Inclusion critera for participation in the study were the presence of HNPGL, biochemical screening of catecholamine excretion in two 24-h urinary samples, plasma sample for CgA analysis and genetic screening for SDH mutations. Exclusion criteria were the use of proton pump inhibitors (25), no radiological assessment in case of increased catecholamine excretion, the presence of paragangliomas in the thorax or abdomen and because renal failure is associated with increased plasma CgA levels, patients with a GFR of less than 60 mL/min were also excluded from the study (26).

All patients were investigated according to structured protocols, which were standard care. These included questions focused on tumor and catecholamine related signs and symptoms, measurement of blood-pressure in the supine position, and after 5 minutes of upright position, in order to screen for orthostatic hypotension. Repetitive head and neck MRI was performed with intervals of at least 2 years. Biochemical screening included the measurement of catecholamine excretion in two 24-h urinary samples: (nor)metanephrines, (nor)epinephrine, vanillylmandelic acid (VMA), dopamine and 3-methoxytyramine. Urine was collected during 24 hours in duplicate under strict dietary regulations (patients abstained from pineapple, avocado, bananas, kiwi, nuts, plums, coffee, tea and other caffeine containing beverages from 2 days preceding and during urine collection) and after withdrawal of medication for at least one week or after changing antihypertensive medication to doxazosin. In order to ascertain adequacy of collection, urinary creatinine excretion was measured as well. Plasma samples for CgA analysis were stored at -80 ºC until analysis.

In case of excessive catecholamine excretion (*i.e.*, any value above the upper reference limit in two urine samples), radiological assessment by MIBG-scans and MRI and/or CT scans of thorax and abdomen was performed to identify the source of excessive catecholamine production.

The study was an evaluation of routine patient care. According to the requirements of the Dutch law, it was not necessary to obtain permission from the institutional ethical commission. Prior to germ line mutation testing, informed consent was obtained from each patient.

Assays

Epinephrine, norepinephrine and dopamine excretion rates in 24-h urine collections were quantified by reversed high pressure liquid chromatography (HPLC) by an electrochemical detector. Inter- and intra-assay coefficients of variations CVs for epinephrine were 4.3-9.0% ranging from high to low levels. For norepinephrine these data are 2.7-3.6% and for dopamine 3.1-4.8%. Vanillylmandelic acid (VMA) excretion in urine was measured using HPLC with fluorometric detection with inter- and intra-assay CVs of 2.4-9.1%. (Nor) metanephrine and 3MT were determined by stable isotope mass fragmentography. The coefficients of variations of the 3-O-methylated catecholamine metabolites (metanephrine, normetanephrine and 3MT) ranged from 1.7 to 4.2% (27). Plasma chromogranin A level was determined by solid phase two-site immunoradiometric assay (CIS Bio International, Gif-sur-Yvette, France).

Reference ranges were obtained in healthy volunteers. These values were for urinary excretion: norepinephrine 0.06-0.47 µmol/24h, epinephrine <0.16 µmol/24h, dopamine 0.46-3.40 µmol/24h, VMA <30 µmol/24h, metanephrine 33-90 µmol/mol creatinine, normetanephrine 64-260 µmol/mol creatinine and 3MT 45-197 µmol/mol creatinine (28), plasma chromogranin < 98 ng/mL. SDH mutation analysis was performed by restriction digestion as described by Taschner *et al.* (7;29).

Data analysis

SPSS for windows version 16.0 (SPSS inc., Chicago, IL) was used for data analysis. Results are expressed as means±standard error (SE), unless specified otherwise. Independent sample t-tests and chi-square tests were used to compare patients with and without increased plasma levels of CgA. The average value of catecholamine excretion rates of two urine samples was used for calculation of p values. Pearson's correlation test was used to study dependence between variables. A p-value <0.05 was considered to represent a significant difference.

Results

Clinical and biochemical data of patients with increased versus normal CgA levels

Plasma CgA levels were increased in 15 of the 95 patients with hereditary HNPGL (16%). Tumor related signs and symptoms (*e.g.*, palpitations, diaphoresis, headache, flushes, dizziness, tinnitus, hearing loss, hoarseness, nausea, vomiting) were not different between patients with increased plasma CgA levels and patients with normal CgA levels (p= 0.26).

Six of these 15 patients (40%) with increased CgA levels had increased 24 hour urinary excretion rates of catecholamines: 2 patients had an elevated urinary excretion rate of norepinephrine, 2 patients of dopamine, 4 patients of VMA, 2 patients of normetanephrine, 3 of metanephrine and 5 patients of 3-methoxytyramine (Table 2). Although none of these patients had increased urinary excretion rates of epinephrine, urinary excretion rates of epinephrine and VMA were significantly higher in the 15 patients with increased plasma CgA levels compared to the patients with normal plasma CgA levels (Table 2).

Twenty-seven of the 80 patients with normal CgA levels (34%) had increased urinary excretion rates of catecholamines: 4 patients had elevated excretion rates of norepinephrine, 5 of dopamine, 7 of VMA, 1 of metanephrine, 1 of normetanephrine and 19 of 3MT. The number of patients with increased urinary catecholamine excretion rates was not significantly different between the patients with increased CgA levels versus the patients with normal CgA levels (40% *vs.* 34%, p=0.77).

Biochemical data of patients with increased versus normal rates of urinary catecholamine excretion

Thirty-three of the 95 patients (35%) had increased urinary excretion of catecholamines. Six of these 33 patients (18%) had increased plasma CgA levels. Nine of the sixty-two patients (15 %) with a biochemically silent tumor (*i.e.*, no increased urinary excretion of catecholamines or their metabolites) had increased CgA levels. Of the 9 patients with increased CgA levels and a biochemical silent tumor, 7 (78%) had glomus caroticum tumors, 5 (56%) glomus vagale tumors, 2 (22%) and 1 (11%) had glomus jugulotympanicum tumors. Mean maximal tumor diameter was 4.4±0.8 cm (range 2-9.7 cm). The number of HNPGL varied from 1 to 4 tumors, the median number of HNPGL was 2. Eight of these patients had SDHD mutations, and 1 patient a SDHB mutation.

Table 1: Clinical characteristics of 95 patients with hereditary HNPGL, with and without increased plasma chromogranine A levels.

	Plasma chromogranin A		
	Normal	Elevated	
Patient characteristics			p-value
Age (yrs) *m* (range)	46 (14-69)	52 (32-78)	0.10
Gender N (%)			0.14
Men	42 (53%)	11 (73%)	
Women	38 (48%)	4 (27%)	
BMI (kg/m^2)	25 ± 0.5	23 ± 1	0.27
Systolic BP (mm Hg)	133 ± 2	136 ± 6	0.54
Diastolic BP (mmHg)	79 ± 1	83 ± 3	0.17
Heart rate (beats/min)	72 ± 1	76 ± 3	0.19
Symptoms/signs	49 (61%)	7 (47%)	0.26
Type of glomustumor			
Caroticum	63 (79%)	11 (73%)	0.74
Jugulare	7 (9%)	3 (20%)	0.19
Vagale	34 (43%)	10 (67%)	0.09
Jugulotympanicum	12 (15%)	1 (7%)	0.69
Tympanicum	6 (8%)	0	0.59
Number of HNPGL			
1	32 (40%)	4 (27%)	0.33
2	24 (30%)	6 (40%)	0.55
3	15 (19%)	4 (27%)	0.49
> 3	9 (11%)	1 (7%)	1.00
SDHx			
SDHD	73 (91%)	13 (87%)	0.63
SDHB	6 (8%)	2 (13%)	0.61
SDHC	1 (1%)	0	1.00

Biochemical and genetic correlations with CgA levels in HNPGL patients

In the 15 patients with an increased CgA level, CgA levels correlated positively with urinary excretion of norepinephrine (r=0.68, p=0.005), normetanephrine (r=0.68, p=0.005) and VMA (r=0.67, p=0.006). In the patients with normal plasma CgA levels, there was a positive correlation between CgA levels and the urinary excretion rate of VMA (r=0.24, p=0.03).

The prevalence of SDHB and SDHD mutations was not different between patients with increased CgA levels and patients with normal CgA levels.

Table 2: Urinary catecholamine excretion rates in 95 HNPGL patients, with and without increased plasma chromogranine A levels.

	Plasma chromogranin A level				
	normal	elevated	normal	elevated	
catecholamines	N tested pos	N tested pos	Mean ± SE	Mean ± SE	p-value
Epinephrine (µmol/24 h)	0	0	0.02 ± 0.0	0.04 ± 0.0	**0.01**
Norepinephrine (µmol/24 h)	4 (5%)	2 (13%)	0.37 ± 0.1	0.63 ± 0.2	0.32
Dopamine (µmol/24 h)	5 (6%)	2 (13%)	1.9 ± 0.1	2.4 ± 0.4	0.24
VMA (µmol/24 h)	7 (9%)	4 (27%)	22.1 ± 0.8	29.6 ± 2.7	**0.02**
Metanephrine (µmol/molcre)	1 (1%)	3 (20%)	51.8 ± 2.5	70.0 ± 8.3	**0.05**
Normetanephrine (µmol/molcre)	1 (1%)	2 (13%)	148 ± 6.5	274 ± 91	0.19
3M-tyramine (µmol/molcre)	19 (24%)	5 (33%)	266 ± 55	761 ± 426	0.27
Chromogranin A (ug/ L)	80	15	58.5 ± 2.1	167 ± 19	**0.00**

A patient is considered to be positive for the urinary excretion of a specific catecholamine or metabolite if the value is above the reference limit in two consecutive urine samples.

Radiological assessment

Radiological studies of the head and neck region were performed in all 95 patients. Radiological evaluation of the head and neck region was performed by MRI in 94% of

patients, and by CT-scanning in 6% of the patients. We refer to Table 1 for further details of the type and number of HNPGL.
There was no significant difference in the number and type of HNPGL between the patients with normal and increased CgA levels. Most patients had carotid body tumors (78%).
Most patients with HNPGL (n=59) had multiple HNPGL, which prohibited the identification of a culprit lesion of CgA secretion. We identified 36 patients with only a single HNPGL. In these 36 patients there was a positive correlation between maximal tumor diameter and plasma CgA concentrations (r=0.57, p=0.001).

Table 3: Plasma CgA levels and urinary catecholamine excretion rates in 95 HNPGL patients with normal and increased urinary catecholamine excretion.

		Urinary catecholamine excretion		
		normal	elevated	
catecholamines	N tested pos	Mean ± SE	Mean ± SE	p-value
Epinephrine (µmol/24 h)	0	0.02 ± 0.0		
Norepinephrine (µmol/24 h)	6	0.36 ± 0.1	1.3 ± 0.5	0.133 ± 0.5
Dopamine (µmol/24 h)	7	1.8 ± 0.1	4.8 ± 0.5	**0.001**
VMA (µmol/24 h)	11	21.3 ± 0.7	38.3 ± 2.7	**0.000**
Metanephrine (µmol/molcre)	4	51.5 ± 2.1	125 ± 12	**0.000**
Normetanephrine (µmol/molcre)	3	146 ± 4.7	828 ± 334	**0.18**
3M-tyramine (µmol/molcre)	24	102 ± 5.0	1062 ± 280	**0.002**
Plasma CgA levels (ug/L)	15	58.5 ± 2.1	167 ± 19	**0.000**

A patient is considered to be positive for the urinary excretion of a specific catecholamine or metabolite if the value is above the reference limit in two consecutive urine samples.

Additional radiological analysis

In the 33 patients with increased urinary excretion of catecholamines and/or their metabolites, abdominal/ thoracic paragangliomas were excluded by MRI in 5 patients, by MRI and MIBG in 13 patients, by CT and MIBG in 1 patient, by MIBG and abdominal MRI in 11 patients, by MIBG alone in 2 patients. In 1 patient abdominal paragangliomas were excluded by MRI.

Discussion

The present study was designed to assess the prevalence of increased CgA levels in patients with HNPGL and the clinical relevance of CgA measurements in these patients. Plasma CgA levels correlated positively with the urinary excretion of norepinephrine, normetanephrine and VMA. Moreover, in patients with a single paraganglioma there was a positive correlation between maximal paraganglioma diameter and plasma CgA levels. Therefore, increased plasma CgA concentrations are associated with increased urinary excretion of catecholamines and their metabolites and tumor size in patients with HNPGL. However, only 16 % of all HNPGL patients, and only 15 % of the patients with biochemically inactive HNPGL had increased plasma CgA concentrations. Therefore, the practical implications of CgA measurements are limited in HNPGL patients.

CgA has been proposed as a tumor marker in patients with pheochromocytomas. The sensitivity to detect these tumors ranges from 83-90% (18;19;22;23;30). However, the sensitivity in patients with paragangliomas is much lower compared to pheochromocytomas, because we observed that only 16% of patients with hereditary HNPGL without evidence of pheochromocytomas had increased plasma CgA levels.

There is a positive correlation between tumor load and plasma CgA concentration (22;31). As patients with head and neck paragangliomas have relatively small tumors, the concentrations of CgA in our patients are relatively low compared to patients with other neuro-endocrine tumors (23). Some of our patients were only identified with HNPGL after a family member was found to have a SDHx mutation. Because family members of an affected individual are screened for HNPGL, these tumors are found in relatively early stages. Therefore, the prevalence of increased CgA levels in patients with HNPGL detected by screening is probably very low. This is in line with the findings of Neumann *et al*., who reported a lower sensitivity of CgA for the detection of pheochromocytoma in patients with hereditary disease compared to patients with sporadic pheochromocytomas (32). The difference in sensitivity can be explained by the fact that these pheochromocytomas were found by screening at earlier stage, when the tumor was smaller.

Nobels *et al*. observed a positive correlation between tumor load and CgA secretion (23). We found no correlation between the number of HNPGL and plasma levels of CgA. This is probably caused by the relatively small size of the HNPGL. Because most patients have more than one tumor, it is difficult to identify a relationship between the size of the tumor and plasma CgA level. We identified 36 patients with only a single HNPGL and in these patients plasma CgA level was positively related with the diameter of the HNPGL, in accordance with the notion put forward by Nobels *et al*.

Chromogranin A (CgA) mediates chromaffin granule biogenesis, is necessary for catecholamine storage and is secreted from neurosecretory vesicles, along with catecholamines (14;15). We found a significant correlation between plasma CgA level and urinary excretion rates of noradrenaline and normetanephrine, but not with urinary dopamine and 3-methoxytyramine excretion. This indicates that increased plasma CgA levels are associated with increased noradrenergic activity, but not with increased dopaminergic activity. This raises the possibility that there are differences between the secretion of norepinephrine versus dopamine by HNPGL in relation to that of CgA. However, at present the precise role of CgA in the monoamine sorting process in the chromaffin cells is still unclear and additional studies are required to elucidate the role of CgA in the sorting and transport of dopamine into granule vesicles.

The patients with increased plasma CgA levels had higher excretion rates of epinephrine and metanephrine compared to the patients with normal plasma CgA levels. Epinephrine is released together with CgA after sympatho-adrenal stimulation. The question erases whether sympatho-adrenal stimulation might be the cause of the higher mean epinephrine and metanephrine excretion in the patients with increased CgA levels. It is known from earlier reports that metanephrine levels increase significantly after physical activity, changes in posture, caffeine or food intake (33;34). Because our patients collected urine under strict dietary regulations, the influence of food and caffeine intake was kept to a minimum. We cannot prevent the influence of physical activity and changes in body posture during urine collection, but we expect these circumstances to occur similarly in both patient groups. Metanephrine levels are also influenced by age and gender (33;35;36). Metanephrine levels are significantly higher in men compared to women, furthermore metanephrine levels increase with age (33). However, there were no differences in age and gender between both groups. Therefore, we cannot simply explain the relation between CgA and catecholamine levels by confounding parameters other than HNPGL.

It has been reported previously that plasma CgA levels are particularly elevated in patients with malignant pheochromocytoma (37). Our cohort consisted of only patients with benign HNPGL as part of genetic syndromes caused by SDHB or SDHD mutations. We found no differences in the prevalence of mutations in the SDHD and SDHB genes between HNPGL patients with increased versus normal CgA levels. Additional studies are required to evaluate the role of CgA as a tumor marker in patients with malignant HNPGL.

In patients with pheochromocytoma, postoperative CgA levels are a good index of the curative outcome of surgery (18;21). Because patients with HNPGL have much smaller tumors and therefore lower plasma CgA levels, it remains questionable whether plasma CgA levels are useful as tumor marker in tracking treatment. Therefore, the role of CgA as a potential tumor marker for follow-up of patients with HNPGL remains to be elucidated.

In conclusion, the measurement of CgA in patients with HNPGL has limited additional value to the combination of radiological and routine biochemical assessment of HNPGL patients.

References

1 DeLellis RA, Lloyd RV, Heitz PU, et al. World Health Organisation classification of tumors of endocrine organs. Oxford, UK: Oxford University Press. 2004.

2 Astuti D, Latif F, Dallol A, et al. (2001) Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet, 69, 49-54.

3 Heutink P, van der Mey AG, Sandkuijl LA, et al. (1992) A gene subject to genomic imprinting and responsible for hereditary paragangliomas maps to chromosome 11q23-qter. Hum Mol Genet, 1, 7-10.

4 Heutink P, van Schothorst EM, van der Mey AG, et al. (1994) Further localization of the gene for hereditary paragangliomas and evidence for linkage in unrelated families. Eur J Hum Genet, 2, 148-58.

5 Rutter J, Winge DR, Schiffman JD. (2010) Succinate dehydrogenase - Assembly, regulation and role in human disease. Mitochondrion, 10, 393-401.

6 Burnichon N, Rohmer V, Amar L, et al. (2009) The succinate dehydrogenase genetic testing in a large prospective series of patients with paragangliomas. J Clin Endocrinol Metab, 94, 2817-27.

7 Bayley JP, van M, I, Weiss MM, et al. (2006) Mutation analysis of SDHB and SDHC: novel germline mutations in sporadic head and neck paraganglioma and familial paraganglioma and/or pheochromocytoma. BMC Med Genet, 7, 1.

8 Bayley JP, Grimbergen AE, van Bunderen PA, et al. (2009) The first Dutch SDHB founder deletion in paraganglioma-pheochromocytoma patients. BMC Med Genet, 10, 34.

9 Bayley JP, Kunst HP, Cascon A, et al. (2010) SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. Lancet Oncol, 11, 366-72.

10 van Houtum WH, Corssmit EP, Douwes Dekker PB, et al. (2005) Increased prevalence of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with SDHD-linked head and neck paragangliomas. Eur J Endocrinol, 152, 87-94.

11 Zak FG, Lawson W. The paraganglionic receptor system. (1982) [1st ed. Springer-Verlag New York Inc.].

12 Erickson D, Kudva YC, Ebersold MJ, et al. (2001) Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. J Clin Endocrinol Metab, 86, 5210-6.

13 van Duinen N, Steenvoorden D, Kema IP, et al. (2010) Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. J Clin Endocrinol Metab, 95, 209-14.

14 Kim T, Tao-Cheng JH, Eiden LE, et al. (2001) Chromogranin A, an "on/off" switch controlling dense-core secretory granule biogenesis. Cell, 106, 499-509.

15 Jain RK, Joyce PB, Gorr SU. (2000) Aggregation chaperones enhance aggregation and storage of secretory proteins in endocrine cells. J Biol Chem, 275, 27032-6.

16 Montesinos MS, Machado JD, Camacho M, et al. (2008) The crucial role of chromogranins in storage and exocytosis revealed using chromaffin cells from chromogranin A null mouse. J Neurosci, 28, 3350-8.

17 Bilek R, Safarik L, Ciprova V, et al. (2008) Chromogranin A, a member of neuroendocrine secretory proteins as a selective marker for laboratory diagnosis of pheochromocytoma. Physiol Res, 57 Suppl 1, S171-S179.

18 d'Herbomez M, Gouze V, Huglo D, et al. (2001) Chromogranin A assay and (131)I-MIBG scintigraphy for diagnosis and follow-up of pheochromocytoma. J Nucl Med, 42, 993-7.

19 d'Herbomez M, Forzy G, Bauters C, et al. (2007) An analysis of the biochemical diagnosis of 66 pheochromocytomas. Eur J Endocrinol, 156, 569-75.

20 Giovanella L, Ceriani L. (2002) Serum chromogranin-alpha immunoradiometric assay in the diagnosis of pheochromocytoma. Int J Biol Markers, 17, 130-4.

21 Grossrubatscher E, Dalino P, Vignati F, et al. (2006) The role of chromogranin A in the management of patients with phaeochromocytoma. Clin Endocrinol (Oxf), 65, 287-93.

22 Hsiao RJ, Neumann HP, Parmer RJ, et al. (1990) Chromogranin A in familial pheochromocytoma: diagnostic screening value, prediction of tumor mass, and post-resection kinetics indicating two-compartment distribution. Am J Med, 88, 607-13.

23 Nobels FR, Kwekkeboom DJ, Coopmans W, et al. (1997) Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. J Clin Endocrinol Metab, 82, 2622-8.
24 Lloyd RV, Sisson JC, Shapiro B, et al. (1986) Immunohistochemical localization of epinephrine, norepinephrine, catecholamine-synthesizing enzymes, and chromogranin in neuroendocrine cells and tumors. Am J Pathol, 125, 45-54.
25 Sanduleanu S, Stridsberg M, Jonkers D, et al. (1999) Serum gastrin and chromogranin A during medium- and long-term acid suppressive therapy: a case-control study. Aliment Pharmacol Ther, 13, 145-53.
26 Tramonti G, Ferdeghini M, Annichiarico C, et al. (2001) Relationship between renal function and blood level of chromogranin A. Ren Fail, 23, 449-57.
27 Kema IP, Meiborg G, Nagel GT, et al. (1993) Isotope dilution ammonia chemical ionization mass fragmentographic analysis of urinary 3-O-methylated catecholamine metabolites. Rapid sample clean-up by derivatization and extraction of lyophilized samples. J Chromatogr, 617, 181-9.
28 Willemsen JJ, Ross HA, Wolthers BG, et al. (2001) Evaluation of specific high-performance liquid-chromatographic determinations of urinary metanephrine and normetanephrine by comparison with isotope dilution mass spectrometry. Ann Clin Biochem, 38, 722-30.
29 Taschner PE, Jansen JC, Baysal BE, et al. (2001) Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes Cancer, 31, 274-81.
30 Canale MP, Bravo EL. (1994) Diagnostic specificity of serum chromogranin-A for pheochromocytoma in patients with renal dysfunction. J Clin Endocrinol Metab, 78, 1139-44.
31 van der Harst E, De Herder WW, de Krijger RR, et al. (2002) The value of plasma markers for the clinical behaviour of phaeochromocytomas. Eur J Endocrinol, 147, 85-94.
32 Neumann HP, Berger DP, Sigmund G, et al. (1993) Pheochromocytomas, multiple endocrine neoplasia type 2, and von Hippel-Lindau disease. N Engl J Med, 329, 1531-8.
33 Deutschbein T, Unger N, Jaeger A, et al. (2010) Influence of various confounding variables and storage conditions on metanephrine and normetanephrine levels in plasma. Clin Endocrinol (Oxf), 73, 153-60.
34 Eisenhofer G, Friberg P, Pacak K, et al. (1995) Plasma metadrenalines: do they provide useful information about sympatho-adrenal function and catecholamine metabolism? Clin Sci (Lond), 88, 533-42.
35 Sawka AM, Thabane L, Gafni A, et al. (2005) Measurement of fractionated plasma metanephrines for exclusion of pheochromocytoma: Can specificity be improved by adjustment for age? BMC Endocr Disord, 5, 1.
36 Williams PD, Puddey IB, Beilin LJ, et al. (1993) Genetic influences on plasma catecholamines in human twins. J Clin Endocrinol Metab, 77, 794-9.
37 Rao F, Keiser HR, O'Connor DT. (2000) Malignant pheochromocytoma. Chromaffin granule transmitters and response to treatment. Hypertension, 36, 1045-52.

Chapter 6

Pheochromocytomas detected by biochemical screening in predisposed subjects are associated with a lower prevalence of clinical and biochemical manifestations and smaller tumors than pheochromocytomas detected by signs and symptoms

N. van Duinen[1], D. Steenvoorden[1], B.A. Bonsing[2], J. Vuyk[3], AHJT Vriends[4], JC Jansen[5,] J.A. Romijn[1], E.P.M. Corssmit[1].

Leiden University Medical Center, [1]Departments of Endocrinology and Metabolic Diseases,
[2]Surgery, [3]Anesthesiology, [4]Center of Human and Clinical Genetics, [5]Otorhinolaryngology, Leiden University Medical Center, Leiden, the Netherlands

The European Journal of Endocrinology 2010 Jul;163 (1):121-127

Abstract

Context: Sporadic pheochromocytomas are detected by clinical signs and symptoms, whereas pheochromocytomas in patients with a known hereditary predisposition for these tumors are detected by repetitive screening for catecholamine excess.

Objective: To document the clinical, biochemical, and pathological differences between patients with sporadic pheochromocytomas detected by signs and symptoms and patients with pheochromocytomas, detected by biochemical screening in established hereditary syndromes

Design: Retrospective follow-up study.

Patients and Methods: We included 60 consecutive patients diagnosed with pheochromocytoma (pheochromocytomas detected by signs and symptoms: n=28, pheochromocytomas detected by screening: n=32) in our center.

Results: Patients with pheochromocytomas detected by screening presented with less complaints of diaphoresis ($p<0.01$), palpitations ($p=0.01$), paleness ($p=0.01$), nausea ($p<0.01$) and vomiting ($p=0.01$) compared to patients with symptomatic pheochromocytomas. Patients with pheochromocytomas detected by screening tended to be younger at the time of diagnosis (41±2 vs. 47±3 years, $p= 0.07$). In addition, patients with pheochromocytomas detected by screening had significantly lower rates of 24-hour urinary catecholamine excretion, and considerably smaller tumors (3.7 ± 0.5 vs. 7.3 ± 0.7 cm, $p<0.01$).

Conclusions*:* Pheochromocytomas detected by screening of patients with a hereditary predisposition have a much lower prevalence of signs and symptoms, lower catecholamine excess and smaller tumors, compared to sporadic pheochromocytomas, detected by signs and symptoms. These data support the benefits of screening for pheochromocytomas in patients with hereditary syndromes predisposing for these tumors.

Introduction

Pheochromocytomas are rare neuroendocrine tumors derived from chromaffin tissue within the adrenal medulla(1). In 12-24% of cases of an apparently sporadic presentation, a pheochromocytoma is caused germline mutations in the von Hippel-Lindau gene (VHL), the RET gene (leading to MEN2), the neurofibromatosis type I gene (NF1) or one of the SDH genes encoding for subunits B, D and C of mitochondrial succinate dehydrogenase (2-8).

Sporadic pheochromocytomas are usually identified by signs and symptoms, including paroxysms of headache, sweating, palpitations and hypertension resulting from the release of catecholamines from the tumor(9). However, a substantial proportion of patients with pheochromocytomas do not have signs and/or symptoms, which carries the risk of unexpected life-threatening catecholamine crises. Therefore, the advice is to screen patients with a known hereditary predisposition for the development of pheochromocytomas at regular intervals by measurement of plasma levels and/or urinary catecholamine excretion rates(10). In case a pheochromocytoma is detected, adrenalectomy is indicated after appropriate pre-operative care (11).

The benefits of screening may be intuitively reasonable, but these have not been formally tested. Therefore, we conducted a retrospective study comparing the data of patients with pheochromocytomas detected by signs and symptoms and of patients with pheochromocytomas, detected by biochemical screening in hereditary syndromes. We compared signs and symptoms, biochemical parameters, (peri)operative outcome and long-term results between these groups.

Patients and methods

We included 60 consecutive patients treated at our center between 1975 and 2008 by adrenalectomy for pheochromocytomas. The Leiden University Medical Center is a tertiary referral center for patients with pheochromocytomas. Of these 60 patients, 28 patients had a sporadic pheochromocytoma, detected because of signs and symptoms of catecholamine excess, whereas 32 patients had a pheochromocytoma detected by screening in subjects with a hereditary predisposition. Hereditary predisposition, which indicated the need for biochemical screening, was defined by a positive family history with phenotypic evidence of a syndrome, predisposing for pheochromocytomas and/or the presence of germline mutations of the RET, VHL, NF1 or SDH genes, respectively. Patients who presented with signs and symptoms, which resulted in the diagnosis and treatment of pheochromocytoma were defined as having sporadic pheochromocytomas. In patients with hereditary pheochromocytomas, clinical follow-up and screening for catecholamine excess was performed at least every two years. In case of excessive catecholamine excretion abdominal MRI and/ or CT scanning was performed.

One patient was incidentally diagnosed with a pheochromocytoma after CT scanning because of a rectal adenoma. The patient had no catecholamine related signs or symptoms, an increased urinary excretion rate of catecholamines and a 5.2 cm tumor in the right

adrenal. Because the aim of the study was to compare the clinical features of patients with pheochromocytomas who present with tumor and/or catecholamine related signs or symptoms versus patients who are screened for pheochromoctyoma we could not classify this patient into one of these categories. Therefore the patient was excluded from the study.

All patients were investigated at the outpatient clinic according to structured clinical protocols, which were standard care. These included questions focused at tumor- and catecholamine related signs and symptoms, measurement of blood-pressure in the supine position, and after 5 minutes of upright position, in order to screen for orthostatic hypotension.

The diagnosis of pheochromocytoma was established by increased urinary excretion rates of catecholamines and/or their metabolites, and confirmed by pathological examination. Radiological evaluation was performed by a combination of ultrasonography, CT, MRI and MIBG scanning. Clinical, endocrinological, radiological, and (peri) operative data were available for all patients.

Prior to surgery the patients were treated with alpha receptor-blocking drugs (doxazosin or phenoxybenzamin) titrated on orthostatic hypotension followed by beta-blockade (propanolol) if necessary (pulse rate >70 bpm during alpha-blockade), and intravenous hydration with isotonic saline the day preceding the operation. Hemodynamic instability during surgery was defined as an episode of systolic arterial pressure above 160 mm Hg and/or a mean arterial pressure <60 mmHg. All episodes were counted and the total duration of hyper/hypotension was recorded. Complications occurring in the pre-, peri- and postoperative periods were recorded. A diagnosis of malignancy was made in case of distant metastases and/or local recurrence. Recurrence was defined as the reappearance of the disease after eradication of the tumor had been confirmed by negative biochemical and imaging tests. The diameter of the tumor was determined on postoperative histopathology.

The study was an evaluation of routine patient care. According to the requirements of Dutch law, it was not necessary to obtain permission from the institutional ethical commission. Prior to germ line mutation testing, informed consent was obtained from each patient.

The screening group comprised 32 patients, 17 with familial paraganglioma syndrome (16 with a SDHD mutation, 1 unknown), 12 with MEN2, 2 with neurofibromatosis and 1 with Von Hippel Lindau disease. In the patients with familial paraganglioma syndrome 14 patients were known with head and neck paragangliomas and were screened for pheochromocytoma, 3 patients were referred for screening because a SDH mutation was found in a family member. In the MEN2 patient group, 11 patients were known with the disease and were screened for pheochromocytoma, 1 patient was screened for pheochromocytoma after his son was diagnosed with MEN2 syndrome and was found to have a tumor. Both patients with neurofibromatosis were diagnosed with pheochromocytoma after they were screened for pheochromocytoma. One patient with a heamangioblastoma of the retina was referred to the department of endocrinology because of suspected Von Hippel Lindau disease. During clinical assessment he was found having a pheochromocytoma.

Assays

Excretion rates of epinephrine, norepinephrine and dopamine in 24 h urine collections were quantified by reversed high pressure liquid chromatography (HPLC) by electrochemical detector. CVs for epinephrine were 4.3-9.0% ranging from high to low levels. For norepinephrine these data were 2.7-3.6% and for dopamine 3.1-4.8%. Vanillylic mandelic acid (VMA) in urine was measured using HPLC with fluorometric detection with inter- and intra-assay CVs of 2.4-9.1%. Reference ranges were obtained in healthy volunteers. These values were for norepinephrine 0.06-0.47 μmol/24h, epinephrine <0.16 μmol/24h, dopamine 0.46-3.40 μmol/24h, and VMA <30 μmol/24h (12). In order to ascertain adequacy of collection, the urinary creatinine content was assessed as well. Urine samples were considered to be positive if 24 hour urinary excretion of catecholamines exceeded the reference limit.

Statistics

SPSS for windows version 16.0 (SPSS inc., Chicago, IL) was used for data analysis. Patients were divided in a sporadic and a hereditary group. Results are expressed as means ± standard error (SE), unless specified otherwise. Independent sample t-tests and chi-square tests were used to compare the data obtained in patients screened for hereditary pheochromocytomas and those in patients with sporadic pheochromocytomas. A p-value <0.05 was considered to represent a significant difference.

Results

Clinical characteristics (Table 1)

Patients with pheochromocytomas detected by biochemical screening, presented with significantly less complaints of diaphoresis, palpitations, paleness, nausea, vomiting and a significantly lower prevalence of type 2 diabetes mellitus, significantly lower systolic blood pressure and significantly lower mean arterial pressure compared to patients with pheochromocytomas detected by signs and symptoms (Table 1). The classical triad of diaphoresis, palpitations and headache was reported by only 5 of 32 patients with pheochromocytomas detected by screening (16%) and by 11 of 28 patients (39%) with sporadic pheochromocytomas (p=0.04 between both groups). Ten patients in the screening group (31%) had no tumor or catecholamine related signs or symptoms. In addition, patients with pheochromocytomas detected by screening tended to be younger at the time of diagnosis.

The mean age of the hereditary paraganglioma patients was 46 ± 3 yrs (range 25- 65 yrs), 36 ± 4 years (range 19- 61 yrs) for the MEN2 patients, 27 ± 11 yrs (16- 38 yrs) for the NF patients and 37 yrs for the patient with Von Hippel Lindau disease.

Time between presentation and surgery was 81 ± 28 days (mean ± SE) in the sporadic group. Time between diagnosis of pheochromocytoma and surgery was 420 ± 162 days (mean ± SE) in the screening group. Patients in the screening group had been observed for

an average duration of 8.4 ± 1.5 years (mean ± SE). In the sporadic group, two patients were diagnosed with a hereditary syndrome after surgery. One patient was diagnosed with MEN2 disease and one patient was diagnosed with neurofibromatosis.

Table 1: Clinical characteristics at the time of diagnosis of patients with sporadic pheochromocytomas versus with pheochromocytomas detected by screening.

	Pheochromocytomas		
Characteristics	**sporadic**	**screening**	**p-value**
Number of patients	28	32	
Gender (n (%))			
Males	14 (50%)	21 (66%)	0.22
Body mass index (kg/m^2)	24.5 ± 0.7	25.3 ± 0.7	0.44
Age at diagnosis (yr)	47 ± 3	41 ± 2	0.07
Symptoms (n(%))			
diaphoresis	21 (75%)	11 (34%)	**<0.01**
palpitations	18 (64%)	9 (28%)	**0.01**
headache	16 (57%)	12 (38%)	0.13
diap. + palp. + head.	11 (39%)	5 (16%)	**0.04**
dizziness	9 (32%)	9 (28%)	0.74
pallor	12 (43%)	4 (13%)	**0.01**
nausea	12 (43%)	1 (3%)	**<0.01**
vomiting	6 (21%)	0	**<0.01**
flushes	2 (7%)	5 (16%)	0.43
Hypertension (>140/90 mmHg)	15 (54%)	11 (34%)	0.13
Type 2 diabetes mellitus (n (%))	5 (18%)	1 (3%)	**0.01**
Systolic blood pressure (mmHg)	156.4 ± 4.7	143.1 ± 2.8	**0.02**
Mean arterial pressure (mm Hg)	116.0 ± 3.5	106.8 ± 2.0	**0.03**
Diastolic blood pressure (mm Hg)	95.7 ± 3.4	88.6 ± 1.9	0.07
Antihypertensive medication (n (%))	13 (46%)	4 (13%)	**<0.01**

Data are shown as mean (SEM), unless mentioned otherwise.

Biochemical results (Table 2)

All patients had increased 24 hour urinary excretion rates of catecholamines and/or their O-methylated metabolites ((nor) epinephrine, dopamine, and/or VMA). Patients with pheochromocytomas detected by biochemical screening had lower rates of urinary excretion of (nor) epinephrine, dopamine and VMA than patients with sporadic pheochromocytomas.

Table 2: Biochemical characteristics of patients with sporadic pheochromocytomas versus pheochromocytomas detected by screening.

	Urinary catecholamine excretion				
	sporadic	**screening**	**sporadic**	**screening**	
catecholamines	N tested pos	N tested pos	Mean ± SE	Mean ± SE	p-value
Creatinine (mmol/ 24h)			12.8 ± 1.1	13.8 ± 0.8	0.44
Epinephrine (µmol/24 h)	17/ 22	8/ 29	1.9 ± 0.5	0.3 ± 0.1	**<0.001**
Norepinephrine (µmol/24 h)	18/ 23	22/ 30	5.5 ± 1.6	1.4 ± 0.3	**0.02**
Dopamine (µmol/24 h)	9/ 21	9/ 29	7.5 ± 2.4	3.1 ± 0.3	0.09
VMA (µmol/24 h)	21/ 23	20/ 30	127.2 ±	42.1 ± 4.2	**<0.01**
Positive urine sample	28/ 28	32/ 32			

Data are shown as mean (SEM), unless mentioned otherwise. VMA= vanillylic mandelic Acid

MIBG- scintigraphy

In 40 patients with 44 pheochromocytomas additional ^{123}MIBG-scanning was performed. Overall, 35 of 44 (80%) adrenal tumors showed increased uptake. Patient based sensitivity of MIBG scanning was 77% in the screening group and 86% in the sporadic group. MIBG uptake was increased in 7/7 of MEN2, 12/16 PGL patients and 1/2 of NF patients. No increased uptake was shown in the patient with Von Hippel Lindau disease. Tumors of patients with a positive MIBG scan tended to be larger than in the patients with a negative scan (3.4±0.6cm *vs.* 2.5±0.7 cm; p=0.08).

Intra- and post-operative events

Laparoscopic surgery was performed in 18% of patients in the sporadic group versus 59% in the screening group. Open surgery was performed in 82% of patients in the sporadic group versus 41% in the screening group. There was no difference in the number of complications between the patients treated with laparoscopic surgery versus open surgery

(p= 1.00). There were no differences in duration of surgery, blood loss and hemodynamic instability between the two groups. Adverse intraoperative events or complications occurred in 9 patients in the patients with pheochromocytomas detected by screening and in 11 patients in the sporadic group (NS). There were no perioperative deaths or cardiovascular events.

Table 3: Peri-operative findings

	Pheochromocytomas		
Intra-operative events	**sporadic**	**screening**	**p- value**
Operation time *m* (min) ± SEM	175 ± 20	167 ± 18	0.78
SAP> 160 mm Hg	21 (75%)	16 (50%)	**0.03**
Episodes *m* ± SEM	4.1 ± 0.8	3.7 ± 0.7	0.68
Duration *m* ± SEM	50.3 ± 10.7	40.7 ± 10.3	0.54
MAP< 60 mm Hg	16 (49%)	11 (34.4%)	0.08
Episodes *m* ± SEM	2.3 ± 0.7	2.6 ± 0.7	0.71
Duration *m* ± SEM	20.4 ± 8.6	29.2 ± 11.1	0.54
Blood loss			
Median *m* (cc)	500	300	

Data are shown as mean (SEM), unless mentioned otherwise.
Legend: SAP= systolic arterial pressure, MAP= mean arterial pressure.

Pathology

In the patients with pheochromocytomas detected by screening, 8 patients (25%) had a pheochromocytoma in the right adrenal gland, 18 (56%) in the left adrenal gland and 6 (19%) had bilateral pheochromocytomas. In the sporadic group, 16 of 28 patients had a pheochromocytoma located in the right adrenal gland (57%), and 10 patients in the left adrenal gland (36%), whereas there was a bilateral pheochromocytoma in 2 patients (7%). Pheochromocytomas in the sporadic group were significantly larger compared to the hereditary group (7.3 ± 0.7 vs. 3.7 ± 0.5 cm, p< 0.01). One patient in the sporadic group had a malignant tumor, reflected by metastatic disease.

Longterm follow-up

Mean follow-up time was 7.5 ± 1.4 years in the patients with pheochromocytomas detected by screening and 7.8 ± 1.4 years in the patients with sporadic pheochromocytomas. In the patients with pheochromocytomas detected by screening, 6 patients (5 with MEN 2A and 1 with hereditary paragangliomas) developed a pheochromocytoma in the opposite adrenal gland, but none developed metastatic or locally recurrent disease. In the patients with sporadic pheochromocytomas, 1 patient developed a second pheochromocytoma, 1 patient metastatic disease and 1 patient local recurrence and metastases. In the sporadic group, 2

patients died of non tumor-related conditions, whereas the cause of death in 1 other patient was unknown.

Discussion

We compared the differences between patients with pheochromocytomas detected by biochemical screening in hereditary syndromes predisposing for pheochromocytomas and patients with sporadic pheochromocytomas (patients with incidentalomas were excluded). Our study clearly shows that patients screened for pheochromocytoma because of a hereditary predisposition presented with less signs and symptoms, lower urinary excretion rates of catecholamines, and smaller tumors than patients presenting with symptomatic pheochromocytomas.

The patients with pheochromocytomas detected by screening had significantly less pheochromocytoma-related complaints. This can be explained by the fact that these pheochromocytomas are found at earlier stage when the tumor is smaller and produces less catecholamines. Our data are in line with those of Pomares *et al.*, who compared patients with sporadic pheochromocytomas and patients with pheochromocytomas caused by the MEN 2A syndrome. They reported similar differences in age at presentation, clinical signs and symptoms, radiological findings, and uni- versus bilateral pheochromocytomas (13). We included patients with pheochromocytomas detected by screening for several hereditary syndromes predisposing to pheochromocytomas (MEN 2A syndrome, NF1, VHL disease, familial paraganglioma syndrome) and patients presenting with sporadic pheochromocytomas. In the patients with pheochromocytomas detected by screening, the age at presentation was 6 years younger than in the sporadic group, which might reflect a benefit of screening and/or earlier development of pheochromocytoma in patients with hereditary disease. The ages of the patients with SDHD related pheochromocytoma found in our study is higher compared with the mean ages reported in the literature. This difference can be explained by the fact that the other studies included children in their studies (2, 4, 14). The mean ages of the patients with MEN2, VHL and NF included in our study are comparable to those reported in the literature (4, 6, 13, 15, 16).The higher age range of our hereditary paraganglioma patients increases the mean age of the hereditary patient group in total.

All our patients had an increased urinary catecholamine excretion. In the patients with pheochromocytomas detected by screening, the mean excretion rates of (nor) epinephrine were significantly lower compared to patients with sporadic pheochromocytomas. In accordance with the study of Eisenhofer *et al.*, who documented a positive relation between the size of the pheochromocytoma and urinary catecholamine excretion, the patients with the sporadic pheochromocytomas had larger pheochromocytomas and higher urinary excretion rates of catecholamines (15).

The reported sensitivity and specificity of MIBG scanning in patients with (suspected) pheochromocytoma ranges from 80- 100%(17-22). MIBG uptake correlates with the production of catecholamines(18). The detection rate of MIBG scanning in the screening group was lower because of the smaller tumors and the lower biochemical activity.

Despite these differences in biochemical activity and the sizes of the pheochromocytomas there were no differences between both groups in perioperative complications. There were no peri-operative deaths, or cardiovascular events. This was probably related to careful pre- and (peri) operative care with careful titration of alpha- and beta blocking drugs. Patients in the screening group were treated more often with laparoscopic surgery. The differences in operation method resulted from differences in tumor diameter (larger in the sporadic group) and because of the innovation with regard to operation technique during the decades. Despite the differences in operation technique, the number of complication was not different between the patients treated with laparoscopic surgery versus open surgery.

Long term follow up revealed additional manifestations of disease in both groups of patients. In the patients with a documented hereditary predisposition several patients developed an additional pheochromocytoma in the contralateral adrenal gland, especially in the case of MEN 2A syndrome (5). In the sporadic group there were several patients with malignant pheochromocytomas. Therefore, long term follow up seems to be warranted in all pheochromocytoma patients, irrespective of initial presentation.

The sporadic group also contained patients with germline mutations predisposing to the development of pheochromocytomas, which were detected by signs and symptoms because they had not been identified previously by prior knowledge of the predisposing hereditary syndrome. However, this does not invalidate our conclusions, because we report the benefits of screening, once this genetic predisposition has been documented.

Because the aim of the study was to compare the clinical features of patients with pheochromocytoma who present with tumor and/or catecholamine related signs or symptoms versus patients who are screened for pheochromoctyoma, we excluded patients who presented with an incidentaloma. Studies comparing the clinical features of patients with incidentalomas versus patients who presented with adrenergic symptoms reported less catecholamine related signs and symptoms, lower plasma catecholamines concentrations, similar tumor size and an older age at presentation in the patient group with incidentalomas (23-25). Because patients with incidentalomas were excluded from the sporadic group in our study, the reported signs and symptoms, the mean urinary catecholamine excretion rate and the mean age at presentation found in our study might be different compared to studies who included patients with incidentalomas.

Patients at risk for developing a pheochromocytoma should be regularly screened. The diagnostic test of choice is the measurement of fractionated plasma and/ or urinary metanephrines (26). Patients with a SDHx mutation, MEN2 disease or Von Hippel- Lindau disease are advised to be screened for pheochromocytoma annually. In case of increased plasma/ urinary catecholamines or their metabolites, additional radiological investigation should be performed to identify the culprit lesion (2, 6, 13, 27). The prevalence of pheochromocytoma is quite low in patients with Neurofibromatosis and therefore screening is not recommended in all patients, but it may be justified in those patients with neurofibromatosis with hypertension, or in those patients who will undergo provocative interventions, such as surgery or pregnancy (7). Children who are predisposed to the development of a pheochromocytoma should be screened annually. The age of initial screening is determined by the specific gene mutation (28).

In conclusion, screening for pheochromocytomas in patients with a hereditary predisposition for these tumors is associated with a much lower prevalence of signs and symptoms, lower values of catecholamine excess and smaller tumors, compared to sporadic pheochromocytomas, detected by signs and symptoms. This observational study supports the usefulness of a screening program for pheochromocytomas in subjects with hereditary predispositions. In addition, we recommend lifelong follow-up in all patients with pheochromocytomas, irrespective of the initial manifestation, because of the risk of developing a new tumor, recurrent or metastatic disease.

References

1 DeLellis RA, Lloyd RV, Heitz PU, Eng C. Pathology and genetisWHO classification of tumours of endocrine organs. Oxford, UK: Oxford university Press. 2004.

2 Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M et al. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 2004 292 943-951.

3 Opocher G, Schiavi F, Conton P, Scaroni C & Mantero F. Clinical and genetic aspects of phaeochromocytoma. Horm Res 2003 59 Suppl 1 56-61.

4 Neumann HP, Bausch B, McWhinney SR, Bender BU, Gimm O, Franke G et al. Germ-line mutations in nonsyndromic pheochromocytoma. N Engl J Med 2002 346 1459-1466.

5 Gagel RF, Tashjian AH, Jr., Cummings T, Papathanasopoulos N, Kaplan MM, DeLellis RA et al. The clinical outcome of prospective screening for multiple endocrine neoplasia type 2a. An 18-year experience. N Engl J Med 1988 318 478-484.

6 Walther MM, Reiter R, Keiser HR, Choyke PL, Venzon D, Hurley K et al. Clinical and genetic characterization of pheochromocytoma in von Hippel-Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma. J Urol 1999 162 659-664.

7 Walther MM, Herring J, Enquist E, Keiser HR & Linehan WM. von Recklinghausen's disease and pheochromocytomas. J Urol 1999 162 1582-1586.

8 Amar L, Bertherat J, Baudin E, Ajzenberg C, Bressac-de PB, Chabre O et al. Genetic testing in pheochromocytoma or functional paraganglioma. J Clin Oncol 2005 23 8812-8818.

9 Lenders JW, Eisenhofer G, Mannelli M & Pacak K. Phaeochromocytoma. Lancet 2005 366 665-675.

10 van Houtum WH, Corssmit EP, Douwes Dekker PB, Jansen JC, van der Mey AG, Brocker-Vriends AH et al. Increased prevalence of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with SDHD-linked head and neck paragangliomas. Eur J Endocrinol 2005 152 87-94.

11 Pacak K, Eisenhofer G, Ahlman H, Bornstein SR, Gimenez-Roqueplo AP, Grossman AB et al. Pheochromocytoma: recommendations for clinical practice from the First International Symposium. October 2005. Nat Clin Pract Endocrinol Metab 2007 3 92-102.

12 Willemsen JJ, Ross HA, Wolthers BG, Sweep CG & Kema IP. Evaluation of specific high-performance liquid-chromatographic determinations of urinary metanephrine and normetanephrine by comparison with isotope dilution mass spectrometry. Ann Clin Biochem 2001 38 722-730.

13 Pomares FJ, Canas R, Rodriguez JM, Hernandez AM, Parrilla P & Tebar FJ. Differences between sporadic and multiple endocrine neoplasia type 2A phaeochromocytoma. Clin Endocrinol (Oxf) 1998 48 195-200.

14 Ricketts CJ, Forman JR, Rattenberry E, Bradshaw N, Lalloo F, Izatt L et al. Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. Hum Mutat 2010 31 41-51.

15 Eisenhofer G, Walther MM, Huynh TT, Li ST, Bornstein SR, Vortmeyer A et al. Pheochromocytomas in von Hippel-Lindau syndrome and multiple endocrine neoplasia type 2 display distinct biochemical and clinical phenotypes. J Clin Endocrinol Metab 2001 86 1999-2008.

16 Patocs A, Karadi E, Toth M, Varga I, Szucs N, Balogh K et al. Clinical and biochemical features of sporadic and hereditary phaeochromocytomas: an analysis of 41 cases investigated in a single endocrine centre. Eur J Cancer Prev 2004 13 403-409.

17 Mannelli M, Ianni L, Cilotti A & Conti A. Pheochromocytoma in Italy: a multicentric retrospective study. Eur J Endocrinol 1999 141 619-624.

18 Milardovic R, Corssmit EP & Stokkel M. Value of I-MIBG Scintigraphy in Paraganglioma. Neuroendocrinology 2009 .

19 Miskulin J, Shulkin BL, Doherty GM, Sisson JC, Burney RE & Gauger PG. Is preoperative iodine 123 meta-iodobenzylguanidine scintigraphy routinely necessary before initial adrenalectomy for pheochromocytoma? Surgery 2003 134 918-922.

20 Mozley PD, Kim CK, Mohsin J, Jatlow A, Gosfield E, III & Alavi A. The efficacy of iodine-123-MIBG as a screening test for pheochromocytoma. J Nucl Med 1994 35 1138-1144.

21 Van Der Horst-Schrivers AN, Jager PL, Boezen HM, Schouten JP, Kema IP & Links TP. Iodine-123 metaiodobenzylguanidine scintigraphy in localising phaeochromocytomas--experience and meta-analysis. Anticancer Res 2006 26 1599-1604.

22 Wiseman GA, Pacak K, O'Dorisio MS, Neumann DR, Waxman AD, Mankoff DA et al. Usefulness of 123I-MIBG scintigraphy in the evaluation of patients with known or suspected primary or metastatic pheochromocytoma or paraganglioma: results from a prospective multicenter trial. J Nucl Med 2009 50 1448-1454.

23 Amar L, Servais A, Gimenez-Roqueplo AP, Zinzindohoue F, Chatellier G & Plouin PF. Year of diagnosis, features at presentation, and risk of recurrence in patients with pheochromocytoma or secreting paraganglioma. J Clin Endocrinol Metab 2005 90 2110-2116.

24 Kopetschke R, Slisko M, Kilisli A, Tuschy U, Wallaschofski H, Fassnacht M et al. Frequent incidental discovery of phaeochromocytoma: data from a German cohort of 201 phaeochromocytoma. Eur J Endocrinol 2009 161 355-361.

25 Noshiro T, Shimizu K, Watanabe T, Akama H, Shibukawa S, Miura W et al. Changes in clinical features and long-term prognosis in patients with pheochromocytoma. Am J Hypertens 2000 13 35-43.

26 Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P et al. Biochemical diagnosis of pheochromocytoma: which test is best? JAMA 2002 287 1427-1434.

27 Havekes B, van der Klaauw AA, Weiss MM, Jansen JC, van der Mey AG, Vriends AH et al. Pheochromocytomas and extra-adrenal paragangliomas detected by screening in patients with SDHD-associated head-and-neck paragangliomas. Endocr Relat Cancer 2009 16 527-536.

28 Waguespack SG, Rich T, Grubbs E, Ying AK, Perrier ND, yala-Ramirez M et al. A Current Review of the Etiology, Diagnosis, and Treatment of Pediatric Pheochromocytoma and Paraganglioma. J Clin Endocrinol Metab 2010 .

Chapter 7

High prevalence of sleep disordered breathing in patients with bilateral carotid body tumors is associated with increased peripheral chemoresponsiveness

N. van Duinen[1], K.W. van Kralingen[2], J.G. van Dijk[3], M.K. Ninaber[2], B. Havekes[4], L. Smid[1], G.J. Lammers[3], J.C. Jansen[5], J.W. Smit[1], J.A. Romijn[1], E.P.M. Corssmit[1]

[1]Departments of Endocrinology and Metabolic Diseases, Pulmonology[2], Neurology[3], Otorhinolaryngology[5], Leiden University Medical Center, Leiden, the Netherlands
[4]Department of Internal Medicine, Maastricht University Medical Center, the Netherlands

Submitted for publication

Abstract

Objective: To assess whether patients with bilateral carotid body resection (bCBR) or with a bilateral carotid body tumor (bCBT) are prone to develop sleep disordered breathing.

Patients and Methods: 9 bCBR patients and 9 bCBT patients were screened for sleep disordered breathing by polysomnography. For every patient a healthy age, sex and BMI matched control person was screened. In addition chemoresponsiveness to CO2 was tested by a rebreathing method. Sleep quality and quality of life were assessed using questionnaires. Daytime and nighttime activity levels were measured using actigraphy.

Results: 6 bCBT patients (67%) suffered from severe sleep apnea syndrome. Sleep apneas were both obstructive and central in nature. bCBT patients had a significantly higher apneu-hypopneu index (AHI) compared to their healthy controls (46.4 ± 12.6 events/ hour sleep vs. 9.7 ± 1.8 events/ hour sleep, p=0.02).We found no differences in the occurrence of mild, moderate and severe sleep disordered breathing between patients with bilateral carotid body resection and their healthy controls. The AHI was positively correlated with an increased peripheral chemoresponsiveness. bCBT patients had a reduced quality of life and a reduced daytime activity level compared to their healthy controls.

Conclusions: Patients with bilateral carotid body tumors are at risk for developing sleep disordered breathing. The development of sleep disordered breathing results from an increase in peripheral chemoreceptor output, causing ventilatory instability. Sleep disordered breathing resulted in an impaired quality of life and a reduced daytime activity level.

Introduction

Paragangliomas are neuro-endocrine tumors, derived from paraganglia (1) Germline mutations in succinate dehydrogenase (SDH) genes cause familial paraganglioma syndromes. Subjects with these SDH mutations have a high prevalence of paragangliomas in the head and neck region. Most frequently, paragangliomas develop in the carotid bodies, which can be surgically removed (2,3)
These carotid bodies have important peripheral chemoreceptor functions for sensing arterial oxygen concentrations and, to a lesser extent, arterial carbon dioxide concentrations [4]. Accordingly, bilateral resection of carotid body paragangliomas dimineshes the ventilatory responsiveness to hypoxia (5). Moreover, after bilateral resection of carotid body paragangliomas, ventilatory sensitivity decreases, resulting in increased resting end-tidal CO_2 pressure (Pet CO_2) values (5). In turn, these high resting PetCO_2 values are associated with irregular breathing, periodic breathing and central sleep apnea (6). Therefore, the question arises whether these patients are prone to develop sleep disordered breathing after bilateral resection of carotid body paragangliomas.
At present, it is unknown whether carotid body paragangliomas in situ cause dysfunction of peripheral chemoreceptor function and if patients with bilateral carotid body paragangliomas have altered chemoreceptor responses. Therefore, the aim of the present study was 1) to assess the prevalence of sleep disordered breathing and 2) to test peripheral chemoreceptor responsiveness in patients with bilateral carotid body paragangliomas and patients after bilateral resection of carotid body paragangliomas.

Patients and methods

Subjects
We included 9 patients with bilateral carotid body paragangliomas (bCBT) and 9 patients who had previously been treated by bilateral resection of carotid body paragangliomas (bCBR). These patients were recruited from the outpatient clinics of the departments of Endocrinology, Otorhinolaryngology and Surgery of the Leiden University Medical Center and through an advertisement on the website of the Dutch paraganglioma patient network. All patients who were willing to participate were invited at our outpatient clinic and screened for suitability for participation in the study. For each patient we recruited a healthy age-, sex- and BMI matched control subject.
Exclusion criteria were sleeping disorders diagnosed by previous polysomnography, psychiatric disorders and/or the use of sleep-influencing medication.
The Medical Ethics Committee of the Leiden University Medical Center approved the study protocol. Written informed consent was obtained from all subjects prior to the study.

Study design
Prior to the study subjects were asked to collect urine during 24 hour in duplicate under strict dietary regulations (patients abstained from coffee, bananas, nuts and alcohol from two days before and during the collection of the urine).

After an overnight polysomnography subjects were admitted to our clinical research center. On the first study day, indirect calorimetry, spirometry and peripheral chemoreflex function tests were performed. In addition, self-reported sleep duration, sleep quality and quality of life were assessed using validated questionnaires (Epworth Sleepiness Scale, St. George Respiratory Questionnaire, Standard Clinical Assessment Questionnaire, The Berlin questionnaire, Pittsburgh sleep quality index, Short form 36, Nottingham Health Profile, Hospital Anxiety and Depression Scale, and the Multidimensional Fatigue Index) [7-15]. Actigraphy (Actiwatch AW7; Cambridge Neurotechnology, Cambridge, UK) was performed to objectively measure habitual sleep duration during 7 days after the study, including one weekend (16).

Polysomnography

Sleep was recorded by a portable polysomnography recorder (Titanium; Embla Systems, Inc., Broomfield, CO). Sleep was visually scored according to the guidelines of the American Association of Sleep Medicine (17) (Iber C 2007 The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. In Ancoli-Israel S., Chesson AL, Quan SF, eds. Westchester. IL: American Academy of Sleep Medicine). The duration of wake, stage I, II, III and REM sleep was assessed. Total sleep duration was the sum of durations of stages I, II, III and REM. Apneas were defined as a cessation of airflow at the nose for longer than 10 seconds. Oxygen saturation was monitored continuously with a pulse oximeter attached to the index finger.

Indirect calorimetry

Basal energy expenditure was measured by indirect calorimetry and a canopy system (Oxygen Pro; CareFusion, Erich Jaeger, Germany). After an overnight fast from 22.00 hrs, measurements started at 10.00 hrs in a quit room at constant room temperature. Subjects were asked to relax and close their eyes without falling asleep while lying supine on a bed with their head in a canopy. The canopy blower was adjusted to maintain the fraction of expired CO_2 below 0.800%. The measurement was performed during 45 minutes. The final 20 minutes were used to calculate Resting Energy Expenditure (REE). .

Spirometry

To assess lung function, inspiratory vital capacity (IVC) and forced expiratory volume in 1 second (FEV1) were determined by spirometry. The FEV1/ IVC ratio was calculated. All lung function parameters were recorded as a percentage of the value predicted for age, sex and length matched controls.

Peripheral chemoreceptor function

To investigate peripheral chemoreflex function, the ventilatory response to hypercapnia was assessed during both normoxic (nVHR) and hyperoxic (hVHR) conditions. We first assumed that the peripheral chemoreflex drive is silenced during hyperoxia. Second, during the normoxic test we assumed that the central and peripheral drives were additive. Since the

additive effect of the peripheral chemoreflex during normoxia is considered 10-20%, the peripheral chemoreflex function will be underestimated in our test (18). Subjects were connected to a closed spirometric circuit while in the sitting position. Partial pressure of carbon dioxide (PCO_2) was monitored continuously at the mouth. End-tidal carbon dioxide ($PetCO_2$) was controlled by adjusting a three way valve, partially short-circuiting the carbon dioxide absorber in the inspiratory limb of the circuit. The nVHR was measured during six minutes. After the subject breathed room air for 10 minutes, the test was repeated under hyperoxic conditions to measure hVHR. To avoid the stimulation of ventilation by 100% oxygen, a PetO2 of approximately 150 mmHg (19). The ventilatory respons to hypercapnia was assessed by a rebreathing method introduced by Read (20). Differences from the classical Read method included use of 4% CO2 concentration and the absence of hyperventilation prior to rebreathing. As a result, a difference occurs between central PCO2 and PetCO2. However, this will diminish slowly over time producing a linear portion of a response line in the second half of the test (21).
The ventilatory respons to hypercapnia was assessed by a rebreathing method (20). Ventilation was plotted against $PetCO_2$. Responsiveness was reported as the slope of the linear regression: $\Delta VE/ PCO_2$.

Assays

Adrenaline, noradrenaline and dopamine excretion in 24 hour urine collections were quantified by reversed high pressure liquid chromatography (HPLC) by an electrochemical detector. CVs for epinephrine were 4.3-9.0% ranging from high to low levels. For norepinephrine these data are 2.7-3.6% and for dopamine 3.1-4.8%. Vanillylic mandelic acid (VMA) excretion in urine was measured using HPLC with fluorometric detection with inter- and intra-assay CVs of 2.4-9.1%. (Nor)metanephrine and 3-methoxytyramine were determined by stable isotope mass spectrometry. The coefficients of variation of the 3-O-methylated catecholamine metabolites (metanephrine, normetanephrine and 3-methoxytyramine) ranged from 1.7 to 4.2% (22).
Reference ranges were obtained in healthy volunteers. These values were norepinephrine: 0.06-3.40 µmol/24h, epinephrine <0.16 µmol/24h, dopamine 0.46-3.40 µmol/24h, VMA <30 µmol/24h, metanephrine 33-90 µmol/mol creatinine, normetanephrine 64-260 µmol/mol creatinine and 3-methoxytyramine 45-197 µmol/mol creatinine (23). SDH mutation analysis was performed by restriction digestion as described by Taschner *et al* (24,25).

Data analysis

SPSS for windows version 16.0 (SPSS inc., Chicago, IL) was used for data analysis. Results are expressed as means ± standard error (SE), unless specified otherwise. Independent sample t-tests and chi-square tests were used to compare patients with their healthy controls. We used the dependant t-test for paired samples to compare the difference

between nVRH and hVHR within the patient and healthy control groups. A p-value <0.05 was considered to represent a significant difference.

Table 2: Clinical characteristics of patients with bilateral carotid body tumors and their matched healthy controls

Characteristics	bCBT N=9	Controls N=9	p-value
Age (yr)	55.6 ± 3.3	53.8 ± 2.7	0.68
Males	56.2 ± 3.5	55.0 ± 2.5	0.79
Females	54.3 ± 8.0	51.3 ± 7.2	0.80
Gender (N)			
Male	6	6	
Female	3	3	
BMI (kg/m^2)	27.4 ± 1.2	26.7 ± 1.3	0.72
Males	27.3 ± 1.1	26.3 ± 1.0	0.51
Females	27.6 ± 3.4	27.7 ± 4.0	0.99
bCBT			
Tumor right (cm)	3.1 ± 1.1 (1.0-9.3)	-	
Tumor left (cm)	3.0 ± 0.8 (0.8-7.2)	-	
Actigraphy			
Daily activity	228 ± 14	280 ± 24	0.07
Nighttime activity	23 ± 3	43 ± 15	0.24
Spirometry *m* (range)			
FEV1 (% Pred)	102 ± 6 (79-132)	108 ± 8 (71-144)	0.58
FEV1/ IVC (% Pred)	96 ± 5 (78-116)*	116 ± 4 (93-125)	<0.01
FEV1/ FVC (%Pred)	82 ± 4 (65-93)	91 ± 2 (80-98)	<0.05

Data are shown as mean values ± SEM, unless specified otherwise.
* p-value< 0.05

Results

Clinical characteristics

We studied 9 bCBT patients and 9 bCBR patients (Table 1 and 2). Mean duration between the date of the resection of second carotid body paraganglioma and the current study was 8.9 ± 2.3 yrs. In the bCBT group one patient had increased 24 hour urinary excretion of 3-methoxytyramine and one patient increased excretion of both 3-methoxytyramine and

norepinephrine. In these patients abdominal/ thoracic paragangliomas were excluded by MRI in both patients. In the bCBR group one patient had increased 24 hour urinary excretion of 3-methoxytyramine. Seven patients in the bCBT group had SDHD mutations, whereas 2 patients were not tested for germline mutations in SDHx genes. In the bCBR group 6 patients had SDHD mutations, whereas 3 patients were not tested. In the bCBT group 6 patients had additional head and neck paragangliomas (HNPGL). Five patients had glomus vagale tumors, one patient a glomus jugulare tumor and one patient a glomus tympanicum tumor. In the bCBR, 3 patients had additional HNPGL. Two patients had glomus vagale tumors and one patient had a glomus jugulare tumor.
There were no differences in RER, REE, VCO_2 and VO_2 between the bCBT patients and their controls and between the bCBR patients and their controls. There was no correlation between RER and REE and the apneu-hypopneu index or with BMI in both patient and control groups.

Questionnaires
Patients with bCBT had reduced quality of life scores compared to their healthy controls. They had worse physical ($p<0.01$) and social functioning ($p<0.05$) and reduced general health perception ($p<0.01$). They reported more complaints of fatigue ($p<0.01$), reduction in activity ($p<0.001$), motivation ($p=0.001$) and worse daytime dysfunction ($p=0.01$) and an increased daytime hyper somnolence ($p=0.05$). bCBR patients reported impaired physical function ($p<0.05$), complaints of general fatigue ($p<0.05$), reduced physical fitness ($p<0.05$) and reported more sleep disturbances ($p<0.05$) compared to the healthy controls.

Sleep apnea
In the patient group with bilateral carotid body tumors (bCBT), 1 patient suffered from mild and 6 patients (67%) suffered from severe sleep disordered breathing. Of these 6 patients, 2 had obstructive sleep apnea (OSA), 2 had central sleep apnea (CSA), 1 had both OSA and CSA and 1 patient had a hypopneu index of 42 events/ hour. In the control group 5 (56%) had mild SDB and one moderate SDB. Patients with bCBT had a higher AHI compared to healthy controls (46.4±12.6 nr/hrs vs. 9.7±1.8 nr/hrs, $p=0.02$) and a higher apnea-index (AI) compared to controls, although this was not significantly different (22.3±9.2 nr/hrs vs. 2.0 ± 1.0 nr/hrs, $p=0.06$). In the patient group with bilateral carotid body resection (bCBR), 3 patients had mild and 1 patient had moderate SDB. There were no differences in AHI, AI and the occurrence of mild, moderate and severe SDB between patients with bCBR and their healthy controls.
Sleep stages
Patients with bCBT had reduced sleep efficiency ($p=0.05$) and spent more time in stage 1 sleep compared to the healthy controls ($p=0.02$). Patients had a significantly higher AHI during non-REM sleep compared to healthy controls (50.2±14.7 vs. 6.1±1.8, $p=0.02$).
In the bCBR group, patients spent more time in sleep stage 1 compared to the healthy controls.

Table 3: The results of polysomnography of 9 bCBT and 9 bCBR patients and their matched healthy controls.

Polysomnography				
	bCBR	Controls	bCBT	controls
Total sleep time (min)	416.5 ± 17.4	442.0 ± 10.0	408.3 ± 17.4	462.4 ± 19.3
Sleep latency (min)	7.7 ± 1.5	4.3 ± 0.6	12.0 ± 5.0	4.7 ± 0.9
Sleep efficiency (%)	89.9 ± 1.2	91.0 ± 1.1	85.8 ± 2.2*	90.8 ± 0.8
% Sleep phase 1	12.7 ± 2.0*	7.6 ± 0.7	12.8 ± 1.9*	7.7 ± 0.7
% Sleep phase 2	38.2 ± 2.9	38.4 ± 2.2	44.1 ± 2.4	40.6 ± 2.2
% Sleep phase 3	24.8 ± 2.1	27.4 ± 2.2	18.7 ± 3.0	25.5 ± 2.3
% REM sleep	24.3 ± 2.5	26.7 ± 1.2	24.5 ± 2.0	26.2 ± 1.1
% awake	8.9 ± 1.3	8.3 ± 1.1	56.7 ± 10.8	39.3 ± 4.8
Awakenings (nr)	23.7 ± 3.7	22.6 ± 3.9	25.0 ± 2.9	22.3 ± 4.0
AH index [1] (nr/h)	6.5 ± 2.3	7.7 ± 1.9	46.4 ± 12.6*	9.7 ± 1.8
Apneu Index (nr/h)	1.2 ± 0.4	1.7 ± 1.0	23.3 ± 9.2	2.0 ± 1.0
Mild n (%)	3 (33%)	4 (44%)	1 (11%)	5 (56%)
Moderate n (%)	1 (11%)	1 (11%)	0	1 (11%)
Severe n (%)	0	0	6 (67%)**	0
AH REM index (nr/h)	11.6 ± 3.9	13.3 ± 5.1	31.3 ± 7.7	18.0 ± 5.6
AH nREM index (nr/h)	4.6 ± 2.5	4.6 ± 1.8	50.2 ± 14.7*	6.1 ± 1.8
AH mean duration (min)	11.0 ± 5.5	10.6 ± 5.5	21.4 ± 2.7	21.7 ± 1.8
Obstructive apneu index (nr/h)	0.8 ± 0.4	0.6 ± 0.5	10.1 ± 4.5	0.9 ± 0.5
Central apneu index (nr/h)	0.4 ± 0.2	1.1 ± 0.8	12.2 ± 6.0	1.1 ± 0.8
Mixed apneu index (nr/h)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Hypopneu index (nr/h)	7.2 ± 3.0	6.0 ± 1.4	23.2 ± 5.3	14.6 ± 7.6

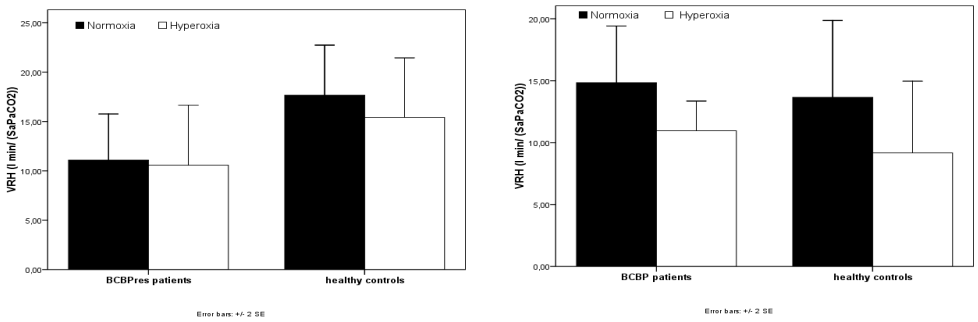
Data are shown as mean values ± SEM, unless specified otherwise.
* p-value< 0.05; ** p-value< 0.01; [1] AH index: Apneu Hypopneu index.

Peripheral chemosensitivity

In the bCBT group, during normoxic conditions, the 1 kPa increase in $PetCO_2$ induced an increase in ventilation of 14.8 ± 2.3 l/min in patients and 13.7 ± 3.1 l/ min in healthy controls (NS) (Figure 1). During hyperoxic conditions, the 1kPa increase in $PetCO_2$ induced an increase in ventilation of 11.0 ± 1.2 l/ min in patients and 9.2 ± 2.9 l/min in healthy controls (NS).

The nVRH and hVHR were significantly different in bCBT patients (14.8 ± 2.3 vs. 11.0 ± 1.2 l/ min, $p<0.05$).
In the bCBR group, during normoxic conditions, the 1 kPa increase in $PetCO_2$ induced an increase in ventilation of 11.1 ± 2.3 l/ min in patients and 17.7 ± 2.5 l/ min in healthy controls (NS). During hyperoxic conditions, the 1kPa increase in $PetCO_2$ induced an increase in ventilation of 10.6± 3.0 l/ min in bCBR patients and 15.4 ± 3.0 l/ min in healthy controls (NS) (Figure 1).

Figure 1: Ventilatory resonses (VR) to CO_2 under normoxic (nVHR) and hyperoxic (hVHR)conditions.



Correlation between Sleep apnea, BMI and ventilatory responses
There was no correlation between BMI and AHI, obstructive apnea index, central apnea index or hypopnea index in both patient groups and control groups. In the patient group with bilateral carotid body tumors, there was no correlation between the occurrence of severe SDB and BMI.
In bCBT patients, there was a highly positive correlation between the apnea-hypopnea index and the difference between nVHR and hVHR ($r=0.97$, $p= 0.001$).

Discussion

The aim of the present study was to assess the prevalence of sleep related complaints by polysomnography in patients with bilateral carotid body paragangliomas and in patients with bilateral resection of carotid body paragangliomas. There was a high prevalence of sleep disordered breathing in patients with bilateral carotid body paragangliomas, but not in patients after bilateral resection of carotid body paragangliomas. Sleep disordered breathing was associated with increased peripheral chemoresponsiveness. Moreover, these subjects have an impaired quality of life and a reduced daytime activity level.
In the past, bilateral resection of carotid bodies was performed in patients with bronchial asthma or COPD to alleviate symptoms. Hypoventilation and apneic spells have been reported as a consequence of this procedure (26,27). The breathing response to hypoxia appeared to be permanently absent after bilateral resection of carotid body paragangliomas, which puts patients at risk of developing sleep disordered breathing (SDB) (5,28).

To assess this risk we performed polysomnography in 9 patients with bilateral resection of carotid body paragangliomas. However, we found no increased prevalence of SDB in the patients with bilateral resection of carotid body paragangliomas compared to their healthy control group, but we did find a high prevalence of SDB in patients with bilateral carotid body paragangliomas. In addition, we found a highly positive correlation between the apneu-hypopneu index and the difference in VHR under normoxic and hyperoxic conditions. This may indicate that the development of SDB is primarily caused by an increase in peripheral chemoreceptor output in patients with bilateral carotid body paragangliomas. The possible role of upper airway obstruction by paragangliomas of the carotid body in association with sleep apnea has been described in a few patients (29,30). In our series the relation between the occurrence of airway narrowing on the MRI scan and the presence of obstructive apneas was equivocal. Two out of the four patients with an OAI of greater than 5 had signs of airway narrowing on static MRI scanning. Further investigations such as dynamic MRI scanning and sleep endoscopy will contribute to the role of airway narrowing in the group of paraganglioma patients.

The ventilatory control system is aimed at supplying oxygen to the body with minimum changes in pH and CO_2. Disturbances in this system result in changes in blood gas tensions in the gas stores of the body and in arterial blood. The peripheral and central chemoreceptors respond to these changes by sending impulses to regulatory centers in the central nervous system, that in turn stimulate the ventilatory muscles to produce a ventilatory effort which counteracts the effect of the disturbances on blood gas tensions (31,32). During wakefulness non-specific environmental stimuli contribute to the tonic activity of muscles that keep the upper airway open. During sleep, the effects of environmental stimulations are reduced, which decreases the activity of the chest wall and the upper airway muscles and affects the resistance and patency of the upper airway. During sleep, chemoreceptors become more important in the regulation of ventilation. The tonic and the phasic respiratory activity of the upper airway muscles increase as chemical drive intensifies. Studies in humans and animals indicate that upper airway muscles frequently have different response characteristics to chemical stimulation than the chest wall muscles [33;34]. There may be a range of stimuli over which the response of the upper airway muscles is less than that of the chest wall. In this range airway obstruction can occur. Obstructive apnea increases the instability of the respiratory system and an unstable system increases the risk of obstructive apneas (35).

Arterial $PaCO_2$ is regulated by negative-feedback control. Fluctuations in $PaCO_2$ stimulate the chemoreflexes to produce ventilatory adjustments to restore $PaCO_2$ back to its original target level. As a result of delays in the feedback and control processes oscillation of the ventilatory control system may arise. The duration and the magnitude of these oscillations are determined by the strength of the chemoreflex response (controller gain) and by the amplification with which the ventilatory changes are translated into changes in $PaCO_2$ (plant gain) (36). The product of controller and plant gain (loop gain) determines the stability of the ventilatory system. The higher the loop gain, the less stable the system and the higher the probability of periodic breathing. Enhanced peripheral chemosensitivity

would increase loop gain and make a subject more prone to develop periodic breathing. Indeed, patients with periodic breathing and idiopathic central sleep apnea have increased central and peripheral chemosensitivity compared to normal controls (37,38). Furthermore, patients with severe obstructive sleep apnea have a more unstable chemical control system compared to patients with mild OSA, which might contribute tot the severity of sleep disordered breathing (39). We found no difference in peripheral chemorespons to CO_2 between patients and controls under normoxic and hyperoxic conditions. However, the patients with bilateral carotid body paragangliomas in situ showed a significantly increased ventilatory response to CO_2 under normoxic conditions compared to hyperoxic conditions, indicating an increased peripheral ventilatory drive. Taken into account that the normoxic test is composed of approximately 10-20% of the peripheral chemoreflex drive, this difference may be more prominent during hypoxic conditions. More importantly, we found a high correlation between the ventilatory response and the apnea-hypopnea index, indicating that an increment in ventilatory response on CO_2 makes a subject more prone to develop SDB.

The question arises whether the increased chemoresponsiveness in the bCBT patients is associated with mutations in the succinate dehydrogenase gene (SDH). Piruat *et al.* have tested whether mice with a partial SDHD deficit have altered carotid body function. They found that the loss of an SDHD allele results in an abnormal enhancement of resting CB activity. This CB overactivity was linked to glomus cell hypertrophy and hyperplasia (40). Therefore, the increased peripheral chemoresponsiveness in the bCBT patients might be the result of hyperplasia of carotid body cells, indicating that tumor formation in the carotid bodies leads to gain of function rather than loss of function.

Intermittent hypoxia can lead to the development of systemic hypertension, heart failure, myocardial infarction and stroke (41). Therefore, it is important to treat sleep disordered breathing. Because of the potential risk of developing sleep disordered breathing, surgery must be considered as an alternative to the general wait-and-scan policy.

Based on these findings all patients with bilateral carotid body tumors should be screened for the presence of sleep disordered breathing.

References

1 DeLellis RA, Lloyd RV, Heitz PU, and Eng C. World Health Organisation classification of tumors of endocrine organs. 2004. Oxford, UK: Oxford University Press.

2 Gardner, P., M. Dalsing, E. Weisberger, A. Sawchuk, and R. Miyamoto. 1996. Carotid body tumors, inheritance, and a high incidence of associated cervical paragangliomas. Am.J.Surg. 172:196-199.

3 Kohler, H. F., A. L. Carvalho, N. V. Mattos Granja, K. Nishinari, and L. P. Kowalski. 2004. Surgical treatment of paragangliomas of the carotid bifurcation: results of 36 patients. Head Neck 26:1058-1063.

4 Zak FG and Lawson W. The paraganglionic chemoreceptor system. [1st ed. Springer-Verlag New York Inc.]. 1982.

5 Timmers, H. J., J. M. Karemaker, W. Wieling, H. A. Marres, H. T. Folgering, and J. W. Lenders. 2003. Baroreflex and chemoreflex function after bilateral carotid body tumor resection. J.Hypertens. 21:591-599.

6 Dempsey, J. A., C. A. Smith, T. Przybylowski, B. Chenuel, A. Xie, H. Nakayama, and J. B. Skatrud. 2004. The ventilatory responsiveness to CO(2) below eupnoea as a determinant of ventilatory stability in sleep. J.Physiol 560:1-11.

7 Ferrer, M., J. Alonso, L. Prieto, V. Plaza, E. Monso, R. Marrades, M. C. Aguar, A. Khalaf, and J. M. Anto. 1996. Validity and reliability of the St George's Respiratory Questionnaire after adaptation to a different language and culture: the Spanish example. Eur.Respir.J. 9:1160-1166.

8 Hays, R. D., C. D. Sherbourne, and R. M. Mazel. 1993. The RAND 36-Item Health Survey 1.0. Health Econ. 2:217-227.

9 Hunt, S. M., S. P. McKenna, J. McEwen, J. Williams, and E. Papp. 1981. The Nottingham Health Profile: subjective health status and medical consultations. Soc.Sci.Med.A 15:221-229.

10 Johns, M. W. 1991. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14:540-545.

11 Monk, T. H., C. F. Reynolds, III, D. J. Kupfer, D. J. Buysse, P. A. Coble, A. J. Hayes, M. A. Machen, S. R. Petrie, and A. M. Ritenour. 1994. The Pittsburgh Sleep Diary. J.Sleep Res. 3:111-120.

12 Netzer, N. C., R. A. Stoohs, C. M. Netzer, K. Clark, and K. P. Strohl. 1999. Using the Berlin Questionnaire to identify patients at risk for the sleep apnea syndrome. Ann.Intern.Med. 131:485-491.

13 Smets, E. M., B. Garssen, B. Bonke, and J. C. De Haes. 1995. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J.Psychosom.Res. 39:315-325.

14 van Kralingen, K. W., K. W. de, G. H. de Groot, B. J. Venmans, B. T. van, A. R. van Keimpema, and P. E. Postmus. 1999. Assessment of sleep complaints and sleep-disordered breathing in a consecutive series of obese patients. Respiration 66:312-316.

15 Zigmond, A. S. and R. P. Snaith. 1983. The hospital anxiety and depression scale. Acta Psychiatr.Scand. 67:361-370.

16 Morgenthaler, T., C. Alessi, L. Friedman, J. Owens, V. Kapur, B. Boehlecke, T. Brown, A. Chesson, Jr., J. Coleman, T. Lee-Chiong, J. Pancer, and T. J. Swick. 2007. Practice parameters for the use of actigraphy in the assessment of sleep and sleep disorders: an update for 2007. Sleep 30:519-529.

17 Iber C The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. In: Ancoli-Israel S, Chesson AL, Quan SF eds. Westchester, IL: American Academy of Sleep Medicine

18 Duffin, J., R. M. Mohan, P. Vasiliou, R. Stephenson, and S. Mahamed. 2000. A model of the chemoreflex control of breathing in humans: model parameters measurement. Respir.Physiol 120:13-26.

19 Becker, H., O. Polo, S. G. McNamara, M. Berthon-Jones, and C. E. Sullivan. 1995. Ventilatory response to isocapnic hyperoxia. J.Appl.Physiol 78:696-701.

20 Read, D. J. 1967. A clinical method for assessing the ventilatory response to carbon dioxide. Australas.Ann.Med. 16:20-32.

21 Jensen, D., G. Mask, and M. E. Tschakovsky. 2010. Variability of the ventilatory response to Duffin's modified hyperoxic and hypoxic rebreathing procedure in healthy awake humans. Respir.Physiol Neurobiol. 170:185-197.

22 Kema, I. P., G. Meiborg, G. T. Nagel, G. J. Stob, and F. A. Muskiet. 1993. Isotope dilution ammonia chemical ionization mass fragmentographic analysis of urinary 3-O-methylated catecholamine metabolites. Rapid sample clean-up by derivatization and extraction of lyophilized samples. J.Chromatogr. 617:181-189.

23 Willemsen, J. J., H. A. Ross, B. G. Wolthers, C. G. Sweep, and I. P. Kema. 2001. Evaluation of specific high-performance liquid-chromatographic determinations of urinary metanephrine and normetanephrine by comparison with isotope dilution mass spectrometry. Ann.Clin.Biochem. 38:722-730.

24 Bayley, J. P., M. van, I, M. M. Weiss, J. C. Jansen, P. H. Oomen, F. H. Menko, B. Pasini, B. Ferrando, N. Wong, L. C. Alpert, R. Williams, E. Blair, P. Devilee, and P. E. Taschner. 2006. Mutation analysis of SDHB and SDHC: novel germline mutations in sporadic head and neck paraganglioma and familial paraganglioma and/or pheochromocytoma. BMC.Med.Genet. 7:1.

25 Taschner, P. E., J. C. Jansen, B. E. Baysal, A. Bosch, E. H. Rosenberg, A. H. Brocker-Vriends, A. G. van Der Mey, G. J. van Ommen, C. J. Cornelisse, and P. Devilee. 2001. Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes.Cancer 31:274-281.

26 Chang, K. C., C. G. Morrill, and H. Chai. 1978. Impaired response to hypoxia after bilateral carotid body resection for treatment of bronchial asthma. Chest 73:667-669.

27 Winter, B. 1973. Carotid body resection. Controversy--confusion--conflict. Ann.Thorac.Surg. 16:648-659.

28 Dahan, A., D. Nieuwenhuijs, and L. Teppema. 2007. Plasticity of central chemoreceptors: effect of bilateral carotid body resection on central CO2 sensitivity. PLoS.Med. 4:e239.

29 Herer, B., F. Royand, E. Kieffer, and J. P. Vincent. 2003. A case report of an obesity hypoventilation syndrome associated with obstructive sleep apnea due to a carotid body paraganglioma. Sleep Med. 4:459-463.

30 Metersky, M. L., R. J. Castriotta, and A. Elnaggar. 1995. Obstructive sleep apnea due to a carotid body paraganglioma. Sleep 18:53-54.

31 Lahiri, S., A. Roy, S. M. Baby, T. Hoshi, G. L. Semenza, and N. R. Prabhakar. 2006. Oxygen sensing in the body. Prog.Biophys.Mol.Biol. 91:249-286.

32 Lopez-Barneo, J., R. Pardal, and P. Ortega-Saenz. 2001. Cellular mechanism of oxygen sensing. Annu.Rev.Physiol 63:259-287.

33 Brouillette, R. T. and B. T. Thach. 1980. Control of genioglossus muscle inspiratory activity. J.Appl.Physiol 49:801-808.

34 Onal, E., M. Lopata, and T. D. O'Connor. 1981. Diaphragmatic and genioglossal electromyogram responses to CO2 rebreathing in humans. J.Appl.Physiol 50:1052-1055.

35 Longobardo, G. S., B. Gothe, M. D. Goldman, and N. S. Cherniack. 1982. Sleep apnea considered as a control system instability. Respir.Physiol 50:311-333.

36 Khoo, M. C. 2000. Determinants of ventilatory instability and variability. Respir.Physiol 122:167-182.

37 Xie, A., B. Wong, E. A. Phillipson, A. S. Slutsky, and T. D. Bradley. 1994. Interaction of hyperventilation and arousal in the pathogenesis of idiopathic central sleep apnea. Am.J.Respir.Crit Care Med. 150:489-495.

38 Xie, A., R. Rutherford, F. Rankin, B. Wong, and T. D. Bradley. 1995. Hypocapnia and increased ventilatory responsiveness in patients with idiopathic central sleep apnea. Am.J.Respir.Crit Care Med. 152:1950-1955.

39 Younes, M., M. Ostrowski, W. Thompson, C. Leslie, and W. Shewchuk. 2001. Chemical control stability in patients with obstructive sleep apnea. Am.J.Respir.Crit Care Med. 163:1181-1190.

40 Piruat, J. I., C. O. Pintado, P. Ortega-Saenz, M. Roche, and J. Lopez-Barneo. 2004. The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia. Mol.Cell Biol. 24:10933-10940.

41 Pack, A. I. and T. Gislason. 2009. Obstructive sleep apnea and cardiovascular disease: a perspective and future directions. Prog.Cardiovasc.Dis. 51:434-451.

Chapter 8

General discussion and summary

General summary and Discussion

Contents

I. Introduction

In recent years the multidisciplinairy approach of the Leiden University Medical Center (LUMC) towards patients with head and neck paragangliomas (HNPGL) has been extended. All patients with HNPGL who consulted the departments of Otorhinolaryngology, Endocrinology and/ or Clinical Genetics were screened for catecholamine excess according to a routine, structured protocol. The aim of inclusion of all consecutive HNPGL patients in the LUMC was to further specify the clinical and biochemical characteristics of these patients. Initial screening consisted of measurement of 24-hour urinary excretion of catecholamines and their O-methylated degradation products in duplicate, which was repeated with intervals of 2 years, if initial biochemical screening was negative. In patients with excessive catecholamine excretion, imaging studies with ^{123}I-MIBG scintigraphy and whole body MRI and/or CT were performed to exclude additional intra- or extra-adrenal non HNPGL.

In this thesis we describe the genetic, biochemical and clinical characteristics of these HNPGL patients. In addition to screening of urinary catecholamine excretion rates, we measured plasma metanephrine levels and compared test sensitivity of these plasma levels with urinary excretion rates of catecholamines. Furthermore, we assessed whether plasma chromogranin A levels has a role as tumor marker in HNPGL patients. A previous study, conducted by B. Havekes *et al* (unpublished data), described an association between glomus caroticum tumors and subjective sleep related complaints. To study these

complaints in more detail we screened patients with bilateral carotid body paragangliomas for sleep disordered breathing.

II. High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands

The hereditary paraganglioma syndrome is caused by mutations in the succinate dehydrogenase (SDH) gene. The SDH gene family (SDHA, SDHB, SDHC and SDHD) encodes the four subunits of complex II of the mitochondrial electron transport chain. SDH contributes to energy metabolism as a component of the tricarboxylic acid cycle, converting succinate to fumarate, and by serving as a source of electrons for mitochondrial respiration, as complex II of the electron transport chain. Because ~30% of patients with apparently sporadic HNPGL are affected by a SDHx mutation, molecular genetic screening should be performed in all patients with HNPGL. The LUMC is a dedicated referral laboratory for paragangliomas in the Netherlands. As almost all DNA samples of Dutch paraganglioma patients are tested in the LUMC, mutation frequency analysis of these patients represents the actual prevalence of SDH gene mutations in the Netherlands. Analysis of 1045 paraganglioma and pheochromocytoma patients and their relatives indicated that the large majority of mutations in SDH subunits or co-factors involve SDHD, followed by SDHB and SDHAF2 mutations, whereas SDHC mutations are extremely rare. The majority of SDH mutation carriers in the Netherlands harbour one specific mutation of SDHD, p.Asp92Tyr (**chapter 2**). This mutation alone accounts for 69% of all mutations in genes encoding subunits of the SDH complex. Several very large families residing in the western part of the Netherlands have been linked to this mutation and a strong founder effect has been demonstrated (1). Compared to the high prevalence of SDHD mutations in The Netherlands (87%), SDHB mutations are strikingly less common (6%). The majority of SDHB mutation carriers also have one of the two known Dutch founder mutations: c.423+1G>A or c.201-4429_287-933del (2;3).

These results differ from international studies in other countries, which show either a two-fold higher frequency of SDHB mutations carriers (4), a two-fold higher frequency of SDHD-related patients (5), or approximately equal numbers (6;7), but none showed the 14-fold higher frequency found in The Netherlands. These differences in mutation prevalences can be explained by the unusual social and demographic history of our country, factors that might have contributed to the prevalence of a notable number of founder mutations in other genes (8). The Netherlands showed high levels of endogamy, marriage within groups, these groups defined by religious, geographic, or linguistic isolation, or a combination of these factors (9). The isolation of communities due to religious barriers to intermarriage was perhaps the most enduring factor, lasting well into the twentieth century, but occupational and geographic isolation were also important factors. These obstacles to intermarriage led to the creation of many genetically isolated populations. Such populations facilitate the proliferation of founder mutations and the well-known Dutch SDHD founder mutation, p.Asp92Tyr, shows a specific geographic focus even today (10). The region harboring the largest p.Asp92Tyr related pedigree also remained Catholic despite the overwhelming

dominance of the Protestant religion in surrounding areas. The prevalence of this specific SDHD mutation is therefore most likely attributable to endogamy, this effect perhaps amplified by limited migration, and rapid population growth in the 20th century (11). The absence of a similar effectin the case of SDHB mutations is probably due to simple chance.

Clinical implications: As mutations of SDHB, SDHC, SDHD and SDHAF2 each result in distinct hereditary paraganglioma syndromes, with differing modes of inheritance, penetrance, risk of pheochromocytoma, and risk of malignant paraganglioma, the identification of the affected gene is essential in providing effective genetic counselling to the individual paraganglioma patient. To date, several algorithms for prioritizing the order of gene-specific mutation testing in paraganglioma patients have been proposed, with the objectives of minimizing mutation screening and cost reduction (12;13). While these algorithms represent a very useful departure point for genetic analysis, it is doubtful whether the effectiveness and outcome of such algorithms is universally applicable, as the *a priori* chance of finding a mutation in a specific gene differs from country to country. Knowledge of these regional differences in the prevalence of mutations will facilitate the tailoring of genetic screening protocols to local circumstances.

III. Biochemical screening of patients with head and neck paragangliomas

Head and neck paragangliomas have the ability to produce and secrete catecholamines. Erickson *et al.* reported that a small proportion (4%) of benign HNPGLs were hyperfunctional (14). In 2005 van Houtum *et al.* documented that the prevalence of catecholamine excess in our SDHD-linked HNPGL patients was much higher than previously appreciated (15). At the time of that study, 15 of 40 consecutive patients (37.5%) had elevated levels of urinary catecholamine excretion. Pheochromocytomas or extra-adrenal paragangliomas were identified in 8 of these 15 patients (20%), indicating that HNPGL may be responsible for excess catecholamine secretion in about 17.5% of the cases included in their study. Importantly, they found no relationship between the levels of catecholamine excess and the complaints generally attributed to catecholamine excess. In **chapter 3** we describe the results of biochemical screening in 136 patients with HNPGLs. Thirty-nine (29%) of the 136 included patients had excessive urinary excretion rates of catecholamines. The majority of the patients with a biochemical active tumor (31 of 136 patients, 23%) had increased urinary excretion of 3-methoxytyramine (3MT), associated with increased dopamine excretion. Patients with 3MT excess had significantly more complaints of palpitations ($p<0.01$), diaphoresis ($p=0.03$), collapse ($p<0.05$) and a higher pulse rate ($p<0.01$). Increased excretion of 3MT was not associated with particular types of HNPGL or genotypes.

It was unknown whether plasma catecholamine levels, including 3MT, were more sensitive parameters of biochemical activity of HNPGL than urinary excretion rates. For the diagnosis of pheochromocytoma, the measurement of plasma free metanephrine levels is the optimal biochemical test with the highest sensitivity and specificity (16). Therefore, in **chapter 4** we studied whether plasma free metanephrines and 3MT levels are more sensitive tests to detect biochemical activity of HNPGL than urinary excretion rates of

catecholamines and 3MT. We screened 124 HNPGL patients for catecholamine excess by measurement of 24-hr urinary excretion rates of (nor)metanephrine, (nor)epinephrine, vanillylmandelic acid (VMA), dopamine and 3MT and plasma levels of (nor)metanephrine and 3MT. Plasma 3MT levels were increased in 35 of the 124 patients (28%), whereas 24-h urinary excretion rates of 3MT were increased in 30 patients (24%) ($p=0.13$). Plasma combined metanephrine levels (NMN, MN, 3MT) were increased in 41 patients (33%), whereas 24-h urinary excretion rates of combined metanephrines were increased in 33 patients (27%, $p<0.05$). The combined assessment of plasma concentrations of free metanephrines and 3MT indicate a higher number of biochemically active HNPGL than the measurement of 24 h urinary excretion rates of combined metanephrines and 3MT. In addition, these data indicate that in HNPGL patients urinary excretion rates of deconjugated 3MT and plasma free 3MT levels do not indentify significantly different numbers of subjects with biochemical active HNPGL.

Clinical implications: Our results indicate that the number of patients with biochemical active HNPGLs is much higher than hitherto appreciated in studies that did not include the measurement of 3MT. We found that only part of the HNPGL patients with increased urinary excretion of 3MT also had increased urinary dopamine excretion. Therefore, urinary 3MT excretion is more sensitive in discovering dopamine-producing HNPGL than urinary dopamine excretion. We observed that the clinical manifestations in patients with increased 3MT excretion were different compared to those in patients with normal excretion of 3MT. Test sensitivity of plasma 3MT measurement equals the measurement of urinary deconjugated 3MT excretion. The combined assessment of plasma metanephrine levels (NMN, MN, 3MT) indicates a higher number of biochemical active tumors tumors than the measurement of 24 hour urinary excretion rates of combined metanephrines and catecholamines.

IV. Chromogranin A as a tumor marker in patients with HNPGL

Although HNPGL have the ability to produce and secrete catecholamines (17;18), we recently demonstrated that only 29% of patients with HNPGL have evidence of increased urinary excretion of catecholamines and/or their metabolites (19). Consequenly, the majority of these patients have biochemical silent tumors and the clinical characteristics of these HNPGL can be evaluated only by imaging techniques. Chromogranin A (CgA) is a secretory protein from neuroendocrine cells that mediates chromaffin granule biogenesis, necessary for catecholamine storage (20;21). CgA is secreted from neurosecretory vesicles, along with catecholamines (22). Plasma CgA is a useful tumor marker in patients with pheochromocytomas (23-29). Increased plasma levels of f chromogranin A have been found in some patients with HNPGL, indicating the presence of secretory granules(30). In **chapter 5** we present the results of the measurement of plasma chromogranin A (CgA) levels in patients with hereditary HNPGLs. Plasma CgA levels were increased in a minority of HNPGL patients, only 16% of all patients had increased plasma CgA levels. In the patients with biochemically inactive tumors, 15% had increased plasma CgA levels.

Therefore the practical implications of the measurement of CgA in HNPGL patients are limited. Interestingly, urinary excretion rates of noradrenaline and normetanephrine were positively related with plasma CgA levels. However, we found no relation between the urinary excretion rates of 3MT and dopamine and plasma CgA levels. This indicates that increased plasma CgA levels are associated with increased noradrenergic activity, but not with increased dopaminergic activity. This might indicate that the secretion of noradrenaline differs from the secretion of dopamine by HNPGL. However, at present the precise role of CgA in the monoamine sorting process in the chromaffin cells is still unclear and additional studies are required to elucidate the role of CgA in the sorting and transport of dopamine into granule vesicles.

V. Pheochromocytomas detected by biochemical screening in predisposed subjects have a different clinical presentation compared to patients detected by signs and symptoms

Pheochromocytomas are rare neuroendocrine tumors derived from chromaffin tissue within the adrenal medulla (31). Pheochromocytomas can be caused by germline mutations in the von Hippel-Lindau gene (VHL), the RET gene (leading to MEN2), the neurofibromatosis type I gene (NF1) or one of the SDH genes encoding for subunits B, D and C of mitochondrial succinate dehydrogenase (32-38). Because of this hereditary predisposition, patients with germline mutations in the VHL, RET, NF1 and SDHx genes are screened for the development of pheochromocytomas. In **chapter 6** we compared the differences in presentation, treatment and longterm follow-up of patients with pheochromocytomas detected by biochemical screening in hereditary syndromes predisposing for pheochromocytomas and patients with sporadic pheochromocytomas. Patients with hereditary tumors presented in an earlier stage of tumor formation with smaller tumors. The levels of catecholamine secretion correlated with tumor diameters. Therefore, patients with hereditary tumors had lower urinary excretion rates of catecholamines which resulted in a lower prevalence of signs and symptoms compared to the patients with sporadic tumors. Despite these differences in biochemical activity and the sizes of the pheochromocytomas there were no differences between both groups in peri-operative complications. This was probably related to careful pre- and (peri-) operative care with careful titration of alpha- and beta blocking drugs. Long term follow up revealed additional manifestations of disease in both groups of patients. In the patients with a documented hereditary predisposition several patients developed an additional pheochromocytoma in the contralateral adrenal gland, especially in the case of MEN 2A syndrome (39). In the sporadic group there were several patients with malignant pheochromocytomas. Therefore, long term follow up seems to be warranted in all pheochromocytoma patients, irrespective of initial presentation.

Clinical implications: Patients at risk for developing a pheochromocytoma should be regularly screened. The diagnostic test of choice is the measurement of fractionated plasma and/ or urinary metanephrines (40). Patients with a SDHx mutation, MEN2 disease or Von Hippel- Lindau disease are advised to be screened for pheochromocytoma every 1-2 years,

depending on the mutation. In case of increased plasma/ urinary catecholamines or their metabolites, additional radiological investigation should be performed to identify the culprit lesion (41-44). The prevalence of pheochromocytoma is quite low in patients with Neurofibromatosis and therefore screening is not recommended in all patients, but it may be justified in those patients with neurofibromatosis with hypertension, or in those patients who will undergo provocative interventions, such as surgery or pregnancy (45). The age of initial screening is determined by the specific gene mutation (46).

VI. Carotid body paragangliomas and sleep related complaints

Quality of life (QoL) studies performed in patients with head and neck paragangliomas reported that HNPGL patients frequently reported fatigue, reduced exercise tolerance and impaired sleep associated with the presence of carotid body glomus tumors (47). To elucidate the pathophysiology between sleep related complaints in patients with carotid body glomus tumors we studied 9 patients with bilateral carotid body tumors (bCBT) and 9 patients with bilateral carotid body resection for sleep disordered breathing by polysomnography (**chapter 7**).There was a high prevalence of sleep disordered breathing in patients with bilateral carotid body paragangliomas, but not in patients with bilateral resection of carotid body paragangliomas. Moreover, bCBT patients reported an impaired quality of life and a reduced daytime activity level compared to healthy controls. Sleep disordered breathing was associated with increased carotid body output, evidenced by an increased pheripheral chemoresponsiveness. The question arises whether the increased chemoresponsiveness in the bCBT patients is associated with mutations in the succinate dehydrogenase gene (SDH). Piruat *et al.* have tested whether mice with a partial SDHD deficit have altered carotid body function. They found that the loss of an SDHD allele results in an abnormal enhancement of resting CB activity. This CB overactivity was linked to glomus cell hypertrophy and hyperplasia (48). Therefore, the increased peripheral chemoresponsiveness in the bCBT patients might be the result of hyperplasia of carotid body cells, indicating that tumor formation in the carotid bodies leads to gain of function rather than loss of function. Intermittent hypoxia can lead to the development of systemic hypertension, heart failure, myocardial infarction and stroke (49). Therefore, it is important to treat sleep disordered breathing. The optimal treatment strategy for bCBT patients with severe sleep apnea remains to be elucidated.

VIII. Summary

The findings of the present thesis can be summarized in the following conclusions:

1. In the Netherlands, the majority of SDHx mutation carriers harbor a mutation in the *SDHD* gene, followed by *SDHB* and *SDHAF2* gene mutations, whereas *SDHC* mutations are extremely rare.

2. Almost 90% of all SDH-related paraganglioma and pheochromocytoma cases in the Netherlands can be attributed to only 6 founder mutations.

3. Twenty- nine percent of patients with head and neck paragangliomas have evidence of a biochemical active tumor. The majority of patients with biochemical active head and neck paragangliomas have increased urinary excretion of 3-methoxytyramine, a metabolite of dopamine.

4. Test sensitivity of plasma 3MT measurement equals the measurement of urinary deconjugated 3MT excretion. The combined assessment of plasma metanephrine levels (NMN, MN, 3MT) indicates a higher number of biochemical active tumors tumors than the measurement of 24h urinary excretion rates of combined metanephrines and catecholamines.

5. Only a minority of HNPGL patients have increased plasma chromogranin A levels. T-herefore, the practical implications of the measurement of plasma CgAlevels are limited in HNPGL patients.

6. Increased plasma CgA levels are associated with increased noradrenergic activity, but not with increased dopaminergic activity. This might indicate that the secretion of noradrenaline differs from the secretion of dopamine in HNPGL.

7. Patients screened for pheochromocytoma, because of a hereditary predisposition, present with less signs and symptoms, lower urinary excretion rates of catecholamines, and smaller tumors than patients presenting with symptomatic pheochromocytomas. Despite these differences in biochemical activity and the sizes of pheochromocytomas there is no difference between patients in peri-operative complications.

8. Patients with bilateral carotid body tumors are at risk for developing sleep disordered breathing. Sleep disordered breathing is associated with increased carotid body output, which is reflected by increased chemosensitivity.

Chapter 9

Samenvatting en discussie

Samenvatting en discussie

Inhoudsopgave

I. Introductie

Enkele jaren geleden is de multidisciplinaire aanpak van het Leids Universitair Medisch Centrum (LUMC) ten aanzien van patiënten met hoofd-hals paragangliomen (head and neck paragangliomas, HNPGL) uitgebreid. Er werd besloten alle HNPGL patiënten die de afdelingen KNO, endocrinologie en/ of klinische genetica consulteerden te screenen op overmatige catecholamine excretie volgens een gestructureerd protocol. Het doel van de inclusie van alle HNPGL patiënten was om de klinische en biochemische karakteristieken van deze patiënten verder te onderzoeken. De initiële screening bestaat uit het bepalen van de uitscheiding van catecholamines en de O-gemethyleerde metabolieten in 24-uurs urine in duplo. Deze screening wordt elke twee jaar uitgevoerd.. Patiënten met een verhoogde catecholamine excretie, ondergaan additioneel onderzoek in de vorm van ^{123}I-MIBG scintigrafie en MRI en/ of CT om additionele (extra-) adrenale paragangliomen uit te sluiten.

In dit proefschrift beschrijven we de genetische, biochemische en klinische karakteristieken van patiënten met hoofd-hals paragangliomen. Als aanvulling op het meten van de catecholamine excretie in de urine, hebben we de plasma concentraties gemeten van de catecholamine metabolieten en vervolgens bepaald welke test de hoogste sensitiviteit behaalde. Verder hebben we de rol van het chromogranine A als tumor marker geëvalueerd in patiënten met HNPGL.
In een eerdere studie, uitgevoerd door Bas Havekes *et al.* werd een verband gelegd tussen glomus caroticum tumoren en slaap gerelateerde klachten. Om deze slaap gerelateerde

klachten verder te specificeren hebben we patiënten met bilaterale glomus caroticum tumoren gescreend op slaapapneusyndroom.

II. Prevalentie van mutaties van succinaat dehydrogenase in Nederland

Hereditair paraganglioom syndroom wordt veroorzaakt door mutaties in het succinaat dehydrogenase (SDH) gen. De familie van SDH genen (SDHA, SDHB, SDHC en SDHD) coderen voor de vier subunits van complex II van de mitochondriële elektron transport keten. SDH is betrokken bij energie metabolisme als component van de citroenzuur cyclus. SDH zet succinaat om in fumaraat en dient als bron voor elektronen tijdens de mitochondriële respiratie, als complex II van de elektron transport keten. Omdat 30% van de patiënten met een veronderstelt sporadisch hoofd-hals paraganglioom een SDHx mutatie hebben, moeten alle patiënten met hoofd hals paragangliomen moleculair genetisch gescreend worden. Het LUMC is een toegewijd verwijs centrum voor patiënten met paragangliomen in Nederland. Omdat bijna alle DNA samples van Nederlandse paraganglioom patiënten in het LUMC geanalyseerd worden, zijn de uitkomsten van deze analyse representatief voor de SDHx mutatie frequentie prevalentie in Nederland. Analyse van 692 patiënten met paragangliomen en feochromocytomen en hun familieleden indiceren dat de meerderheid van de mutaties in SDH subunits of cofactoren gelegen zijn in het SDHD gen, gevolgd door het SDHB en het SDHAF2 gen mutaties, terwijl mutaties in het SDHC gen zeer zeldzaam zijn. De meerderheid van de SDH mutatie dragers in Nederland heeft één specifieke mutatie in het SDHD gen, de p.Asp92Tyr mutatie (**hoofdstuk 2**). Deze mutatie komt voor bij 69% van alle SDH mutatie dragers. Verscheidene grote families, woonachtig in het westen van Nederland zijn in verband gebracht met deze mutatie en een sterk ‘founder’ effect is aangetoond (1). Vergeleken met de hoge prevalentie van SDHD mutaties in Nederland (87%), komen SDHB mutaties veel minder vaak voor (6%). De meerderheid van de SDHB mutatie dragers heeft ook één van de twee bekende Nederlandse founder mutaties: c.423+1G>A of c.201-4429_287-933del (2;3).

Deze resultaten verschillen van de uitkomsten van andere internationale studies. Deze laten of een tweevoudig hogere frequentie van SDHB mutatie dragers (4), of een tweevoud hogere frequentie van SDHD mutatie dragers (5), of ongeveer gelijke aantallen (6;7). Echter, geen van deze studies laat een 14-voudig verschil zien, zoals in Nederland. Dit verschil in mutatie prevalentie kan worden verklaard door de ongewone sociale en demografische geschiedenis van ons land, factoren die hebben bijgedragen aan de prevalentie van een opmerkelijk aantal founder mutaties in andere genen (8). In Nederland was er een aanzienlijke endogamie, huwelijk binnen groepen. Deze groepen werden gedefinieerd door religieuze, geografische of linguïstische isolatie of door een combinatie van deze factoren (9). De isolatie van gemeenschappen, vanwege religieuze barrières, was waarschijnlijk de meest langdurige factor, welke voortdurende tot in de twintigste eeuw, maar beroeps- of geografische isolatie waren ook belangrijke factoren. Dit obstakel tegen gemengde huwelijken leidde tot genetisch geïsoleerde populaties. Deze populaties

faciliteren de proliferatie van founder mutaties en de welbekende Nederlandse founder mutatie p.Asp92Tyr, laat ook tegenwoordig nog een specifieke geografische focus zien (10). Het gebied dat de grootste p.Asp92Tyr gerelateerde familie laat zien bleef ook katholiek, ondanks de aanzienlijke dominantie van het protestantisme in de omliggende gebieden. De prevalentie van deze specifieke SDHD mutatie is daarom waarschijnlijk het gevolg van endogamie en dit effect is waarschijnlijk vergroot door de gelimiteerde migratie en snelle groei van de populatie in de 20e eeuw (11). De afwezigheid van dit effect bij SDHB dragers is waarschijnlijk het effect van kans.

Klinische implicaties: Omdat SDHB, SDHC, SDHD en SDHAF2 mutaties elk resulteren in een verschillend fenotype, met verschillende wijzen van overerving, penetrantie, verschillende risico's op feochromocytomen, en op maligne paragangliomen, is de identificatie van het aangedane gen essentieel in het bieden van adequate genetische counseling aan de individuele patiënt met paragangliomen. Er bestaan verschillende algoritmes om de juiste volgorde van testen van gen mutaties te bepalen, met het doel het testen van mutaties zo effectief en kosten besparend te verrichten (12;13). Deze algoritmes zijn zeer nuttig bij het bepalen van het traject van mutatie testen, maar deze algoritmes zijn niet zonder meer universeel toepasbaar, omdat de *a priori* kans op het vinden van een mutatie in een specifiek gen verschilt van land tot land. Kennis van regionale verschillen in de prevalentie van deze mutaties zal het opstellen van screenings protocollen toegepast op een specifieke regio faciliteren.

III. Biochemische screening van patiënten met hoofd-hals paragangliomen

Hoofd-hals paragangliomen (HNPGL) produceren catecholamines. Erickson *et al.* rapporteerden dat 4% van de benigne HNPGL hyperfunctioneel waren. In 2005 concludeerden van Houtum *et al.* dat de prevalentie van catecholamine producerende paragangliomen in onze SDHD-gerelateerde HNPGL patiënten veel hoger was dan eerder werd verondersteld (15). In zijn studie werd gerapporteerd dat 15 van de 40 patiënten (37.5%) een verhoogde uitscheiding van catecholamines in de urine had. Een feochromocytoom of (extra-) adrenaal paraganglioom werd aangetoond in 8 van deze 15 patiënten (20%). Dit indiceerde dat HNPGL verantwoordelijk zouden zijn voor een verhoogde urinaire catecholamine excretie in ongeveer 17.5% van de geïncludeerde casussen. Het is belangrijk te vermelden is dat er geen associatie was tussen de mate van catecholamine excretie en klachten die veroorzaakt zouden kunnen worden door catecholamine exces.

In **hoofdstuk 3** beschrijven we de resultaten van de biochemische screening in een veel grotere serie van patiënten: 136 patiënten met HNPGL. Negenendertig (29%) van de 136 geïncludeerde patiënten had een verhoogde catecholamine excretie in de urine. De meerderheid van de patiënten met een biochemisch actieve tumor (31 van de 136 patiënten, 23%) had een verhoogde excretie van 3-methoxytyramine (3MT) in de urine, geassocieerd met een verhoogde dopamine excretie. Patiënten met een verhoogde 3MT excretie hadden significant meer klachten van palpitaties, zweten, collaps en een hogere polsfrequentie. Een

verhoogde 3MT excretie was niet geassocieerd met een specifiek type HNPGL of een specifiek genotype.

Het meten van plasma metanefrine concentraties is de optimale biochemische test met de hoogste sensitiviteit en specificiteit voor het aantonen van een feochromocytoom (16). In **hoofdstuk 4** bespreken we de testgevoeligheid van plasma vrije metanefrines, inclusief 3MT, versus de excretie van catecholamines en metanefrines in de urine om een biochemisch actieve HNPGL aan te tonen. We screenden 124 HNPGL patiënten op een verhoogde catecholamine secretie door het meten van (nor)metanefrine, (nor)adrenaline, vanillylmandelic acid (VMA), dopamine en 3MT in 24-uurs urine en (nor)metanefrine en 3MT in plasma. Plasma 3MT concentraties waren verhoogd in 35 van de 124 patiënten (28%) en de excretie van 3MT in 24-uurs urine was verhoogd in 30 patiënten (24%) ($p=0.13$). Gecombineerde plasma metanefrine concentraties (NMN, MN, 3MT) waren verhoogd in 41 patiënten (33%), terwijl deze parameters bij 33 patiënten in de 24-uurs urine verhoogd waren (27%, $p<0.05$). De beoordeling van plasma concentraties van de vrije metanefrines en 3MT indiceren een hoger aantal biochemisch actieve HNPGL dan het meten van de gedeconjugeerde metabolieten in 24-uurs urine. Onze data tonen aan dat het meten van gedeconjugeerd 3MT in de 24 uurs urine en het vrije 3MT in plasma geen significant verschil in aantal biochemische actieve HNPGL aantonen.

Klinische implicaties: onze resultaten indiceren dat het percentage HNPGL patiënten met een biochemisch actieve tumor aanzienlijk hoger is dan werd vermeld in eerdere studies, die het 3MT niet beoordeelden. Wij toonden aan dat slechts een klein deel van de patiënten met een verhoogde 3MT excretie in de urine ook een verhoogde dopamine excretie in de urine hadden. Daarom is de meting van 3MT in de urine een sensitievere marker voor het aantonen van een dopamine producerend paraganglioom dan de meting van dopamine excretie in de urine. We zagen een verschil in klinische manifestaties tussen patiënten met een verhoogd 3MT en een normaal 3MT. De test sensitiviteit van plasma vrij 3MT is gelijk aan de sensitiviteit van het meten van gedeconjugeerd 3MT in de urine. Het meten van de plasma vrije metanefrines (NMN, MN, 3MT) indiceert een hoger aantal biochemisch actieve HNPGL vergeleken met het meten van deze metabolieten in gedeconjugeerde vorm en catecholamines in 24-uurs urine.

IV. Chromogranine A als tumor marker voor patiënten met hoofd-hals paragangliomen

Hoewel HNPGL catecholamines kunnen produceren en uitscheiden (17;18), toonden we recent aan dat slechts 29% van deze patiënten een verhoogde catecholamine excretie hadden in 24-uurs urine (19). De meerderheid van deze patiënten heeft dus een biochemisch inactieve tumor en de klinische karakteristieken van deze HNPGL patiënten kan alleen met behulp van beeldvormend onderzoek aangetoond worden. Chromogranine A is een secretoir proteïne afkomstig uit neuro-endocriene cellen dat de chromaffiene granuul biogenese faciliteert, belangrijk voor de opslag van catecholamines. CgA wordt samen met

de catecholamines uit neurosecretoire blaasjes gesecerneerd (22). Plasma CgA is een bruikbare tumor marker voor patiënten met feochromocytomen (23-29). Verhoogde plasma levels van chromogranine A zijn aangetoond bij enkele patiënten met HNPGL, hetgeen past bij de aanwezigheid van secretoire granules in HNPGL (30). In **hoofdstuk 5** presenteren we de resultaten van de metingen van plasma CgA in patiënten met erfelijke HNPGL. Plasma CgA concentraties waren verhoogd in slechts een klein deel van de patiënten met HNPGL: slechts 16% van alle patiënten had een verhoogd plasma CgA. Van de patiënten met een biochemisch inactieve tumor had slechts 15% een verhoogd plasma CgA concentratie. Daarom zijn de praktische implicaties van de bepaling van plasma CgA spiegels bij patiënten met HNPGL beperkt. Er was een positieve correlatie tussen de urine excretie ratio's van noradrenaline en normetanefrine en plasma CgA concentraties. We vonden echter geen associatie tussen urine excretie van 3MT en dopamine enerzijds en plasma CgA concentraties anderzijds. Dit betekent dat verhoogde plasma CgA concentraties geassocieerd zijn met een verhoogde noradrenerge activiteit, maar niet met een verhoogde dopaminerge activiteit. Dit zou betekenen dat de secretie van noradrenaline verschilt van de secretie van dopamine uit HNPGL. Echter, de precieze rol van chromogranine A in het monoaminen sorterings proces in de chromaffiene cellen blijft nog steeds onduidelijk en verder onderzoek is nodig om de rol van CgA in het sorteren en transporteren van dopamine in de secretoire granules uit te zoeken.

V. Feochromocytomen opgespoord met biochemische screening van patiënten met een genetische predispositie presenteren zich anders dan sporadische feocromocytomen die ontdekt worden bij patiënten met symptomen

Feochromocytomen zijn zeldzame neuro-endocriene tumoren afkomstig van chromaffien weefsel uit de adrenale medulla (31). Een feochromocytoom kan veroorzaakt worden door kiemcel mutaties in het Von-Hippel Lindau gen (VHL), het RET gen (MEN2), het neurofibromatose type I gen (NF1), of één van de SDH genen die coderen voor subunits B, D en C van het mitochondriale succinaat dehydrogenase (32-38). Vanwege deze erfelijke predispositie, worden patiënten met kiemcel mutaties in de VHL, RET, NF1 en SDHx genen gescreend op de ontwikkeling van een feochromocytoom. In **hoofdstuk 6** vergelijken we de verschillen in presentatie, behandeling, en langdurige follow-up van patiënten met feochromocytomen als gevolg van een erfelijk syndroom opgespoord met biochemische screening vergeleken met patiënten met sporadische feochromocytomen. Patiënten met erfelijke tumoren presenteerden zich in een eerder stadium van tumor vorming met kleinere tumoren. De mate van catecholamine excretie is gecorreleerd aan tumor diameter. Daarom hadden de patiënten met erfelijke tumoren een lagere catecholamine excretie in de urine en presenteerden zij zich met minder symptomen dan de patiënten met sporadische tumoren. Ondanks deze verschillen in biochemische activiteit en de grootte van de tumoren was er geen verschil tussen beide groepen ten aanzien van perioperatieve complicaties. Waarschijnlijk was dit het gevolg van nauwkeurige pre- en (peri-)operatieve zorg met nauwkeurige titratie van alfa- en beta-receptor blokerende medicatie. Langdurige follow-up toonde additionele manifestaties van de ziekte aan in

beide groepen patiënten. In de patiëntengroep met een aangetoonde erfelijke predispositie ontwikkelden verscheidene patiënten een feochromocytoom in de contralaterale bijnier, voornamelijk bij de patiënten met MEN 2A syndroom (39). In de sporadische groep waren er verscheidene patiënten met een maligne feochromocytoom. Langdurige follow-up is geïndiceerd van alle patiënten met feochromocytomen, ongeacht de initiële presentatie.

Klinische implicaties: patiënten met een verhoogd risico op het ontwikkelen van een feochromocytoom moeten regelmatig gescreend worden. De diagnostische test van keuze is het meten van de gefractioneerde metanefrines in plasma en/ of urine (40). Patiënten met een SDHx mutatie, MEN 2A of Von-Hippel Lindau worden geadviseerd zich om de één of twee jaar te laten screenen (termijn is afhankelijk van het type mutatie) op de ontwikkeling van een feochromocytoom. In het geval van een verhoogde catecholamine excretie in plasma of urine, moet er aanvullend beeldvormend onderzoek verricht worden om de laesie op te sporen (41-44). De prevalentie van feochromocytomen is laag bij patiënten met neurofibromatose en daarom wordt screening niet geadviseerd aan alle patiënten, maar is screening wel gerechtvaardigd in die patiënten met hypertensie of degenen die provocatieve interventies ondergaan, zoals chirurgie of zwangerschap (45). De leeftijd waarop screening gestart moet worden wordt bepaald door de specifieke gen mutatie (46).

VI. Glomus caroticum tumoren en slaap gerelateerde klachten

Kwaliteit van leven studies uitgevoerd onder patiënten met hoofd-hals paragangliomen rapporteerden dat HNPGL patiënten frequent klachten hebben van vermoeidheid, gereduceerde inspanningstolerantie en een verstoorde slaap welke gerelateerd zijn aan de aanwezigheid van glomus caroticum tumoren (47). Om de relatie tussen slaap gerelateerde klachten bij patiënten met glomus caroticum tumoren nader te analyseren, onderzochten we 9 patiënten met bilaterale glomus caroticum tumoren (bCBT) en 9 patiënten met een bilaterale resectie van het glomus caroticum (bCBR) op slaap apneu syndroom met behulp van polysomnografie (**hoofdstuk 7**). De prevalentie van het slaap-apneu syndroom was hoog onder de patiënten met bilaterale glomus caroticum tumoren, maar niet onder de patiënten met een bilaterale glomus caroticum resectie. Verder rapporteerden de bCBT patiënten een verminderde kwaliteit van leven en een gereduceerd activiteitsniveau gedurende de dag vergeleken met gezonde controles. De aanwezigheid van het slaap apneu syndroom bleek geassocieerd te zijn met een verhoogde output van het glomus caroticum, wat tot uiting kwam in een toegenomen chemosensitiviteit. De vraag doemt op of een toegenomen chemosensitiviteit in de bCBT patiënten het gevolg is van een mutatie in het succinaat dehydrogenase gen (SDH). Piruat *et al.* onderzochten of muizen met een partieel SDH defect een verandering in glomus caroticum activiteit hadden. Zij toonden aan dat het verlies van een SDHD allel resulteerde in een abnormale toename van de activiteit van het glomus caroticum in rust. Deze glomus caroticum hyperactiviteit werd geassocieerd met glomus cel hypertrofie en hyperplasie (48). De toegenomen perifere chemosensitiviteit in de bCBT patiënten zou het resultaat kunnen zijn van hyperplasie van de cellen van het glomus caroticum, wat indiceert dat tumor formatie in de glomus carotica leidt tot een

toename van de CB activiteit in plaats van een afgenomen activiteit. Intermitterende hypoxie kan leiden tot de ontwikkeling van systemische hypertensie, hartfalen, myocard infarct en beroertes (49). Daarom is het belangrijk om slaap apneu syndroom te behandelen. De optimale behandelings methode van slaap apneus bij bCBT patiënten moet nog onderzocht worden.

VII. samenvatting

Mijn proefschrift heeft de volgende conclusies opgeleverd:

I. In Nederland heeft de meerderheid van de SDHx mutatie dragers een mutatie in het SDHD gen, gevolgd door SDHB en SDHAF2 gen mutaties. SDHC mutaties zijn zeer zeldzaam.

II. Bijna 90% van alle SDH-gerelateerde paragangliomen en feochromocytomen casussen in Nederland worden veroorzaakt door slechts 6 founder mutaties.

III. Negentwintig procent van de patiënten met hoofd-hals paragangliomen hebben aanwijzingen voor een biochemisch actieve tumor. De meerderheid van de patiënten met een biochemisch actieve tumor hebben een verhoogde excretie van 3-methoxytyramine, een metaboliet van dopamine, in de urine.

IV. De test sensitiviteit van plasma vrij 3MT is gelijk aan de test sensitiviteit van gedeconjugeerd 3MT in 24-uurs urine. De meting van de gecombineerde plasma vrije metanefrines (NMN, MN, 3MT) toont een hoger aantal biochemisch actieve tumoren aan dan het meten van de gecombineerde gedeconjugeerde metanefrines en catecholamines in 24-uurs urine.

V. Slechts een klein deel van de patiënten met hoofd-hals paragangliomen heeft een verhoogde plasma chromogranine A spiegel. Daarom zijn de praktische implicaties van de meting van plasma chromogranine A concentraties in patiënten met HNPGL beperkt.

VI. Een verhoogde plasma chromogranine A concentratie is geassocieerd met een toegenomen nor-adrenerge activiteit, maar niet met een toegenomen dopaminerge activiteit. Dit indiceert dat de secretie van noradrenaline verschilt van de secretie van dopamine door HNPGL.

VII. Patiënten met een erfelijke predispositie die gescreend worden op een feochromocytoom, presenteren zich met minder symptomen, lagere catecholamine excretie in de urine en kleinere tumoren vergeleken met patiënten die zich presenteren met symptomatische feochromocytomen. Ondanks de verschillen in

biochemische activiteit en de grootte van de feochromocytomen is er geen verschil tussen de patiënten in perioperatieve complicaties.

VIII. Patiënten met bilaterale glomus caroticum tumoren hebben een verhoogd risico op slaap apneu syndroom. Bij deze patiënten is dit slaap apneu syndroom geassocieerd met een toegenomen activiteit van het glomus caroticum, wat tot uiting komt in een toegenomen chemosensitiviteit.

References

1 van Schothorst EM, Jansen JC, Grooters E, Prins DE, Wiersinga JJ, van der Mey AG, van Ommen GJ, Devilee P, Cornelisse CJ 1998 Founder effect at PGL1 in hereditary head and neck paraganglioma families from the Netherlands. Am J Hum Genet 63:468-473

2 Bayley JP, Grimbergen AE, van Bunderen PA, van der WM, Kunst HP, Lenders JW, Jansen JC, Dullaart RP, Devilee P, Corssmit EP, Vriends AH, Losekoot M, Weiss MM 2009 The first Dutch SDHB founder deletion in paraganglioma-pheochromocytoma patients. BMC Med Genet 10:34

3 Bayley JP, van M, I, Weiss MM, Jansen JC, Oomen PH, Menko FH, Pasini B, Ferrando B, Wong N, Alpert LC, Williams R, Blair E, Devilee P, Taschner PE 2006 Mutation analysis of SDHB and SDHC: novel germline mutations in sporadic head and neck paraganglioma and familial paraganglioma and/or pheochromocytoma. BMC Med Genet 7:1

4 Benn DE, Gimenez-Roqueplo AP, Reilly JR, Bertherat J, Burgess J, Byth K, Croxson M, Dahia PL, Elston M, Gimm O, Henley D, Herman P, Murday V, Niccoli-Sire P, Pasieka JL, Rohmer V, Tucker K, Jeunemaitre X, Marsh DJ, Plouin PF, Robinson BG 2006 Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. J Clin Endocrinol Metab 91:827-836

5 Mannelli M, Castellano M, Schiavi F, Filetti S, Giacche M, Mori L, Pignataro V, Bernini G, Giache V, Bacca A, Biondi B, Corona G, Di TG, Grossrubatscher E, Reimondo G, Arnaldi G, Giacchetti G, Veglio F, Loli P, Colao A, Ambrosio MR, Terzolo M, Letizia C, Ercolino T, Opocher G 2009 Clinically guided genetic screening in a large cohort of italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas. J Clin Endocrinol Metab 94:1541-1547

6 Neumann HP, Erlic Z, Boedeker CC, Rybicki LA, Robledo M, Hermsen M, Schiavi F, Falcioni M, Kwok P, Bauters C, Lampe K, Fischer M, Edelman E, Benn DE, Robinson BG, Wiegand S, Rasp G, Stuck BA, Hoffmann MM, Sullivan M, Sevilla MA, Weiss MM, Peczkowska M, Kubaszek A, Pigny P, Ward RL, Learoyd D, Croxson M, Zabolotny D, Yaremchuk S, Draf W, Muresan M, Lorenz RR, Knipping S, Strohm M, Dyckhoff G, Matthias C, Reisch N, Preuss SF, Esser D, Walter MA, Kaftan H, Stover T, Fottner C, Gorgulla H, Malekpour M, Zarandy MM, Schipper J, Brase C, Glien A, Kuhnemund M, Koscielny S, Schwerdtfeger P, Valimaki M, Szyfter W, Finckh U, Zerres K, Cascon A, Opocher G, Ridder GJ, Januszewicz A, Suarez C, Eng C 2009 Clinical predictors for germline mutations in head and neck paraganglioma patients: cost reduction strategy in genetic diagnostic process as fall-out. Cancer Res 69:3650-3656

7 Erlic Z, Rybicki L, Peczkowska M, Golcher H, Kann PH, Brauckhoff M, Mussig K, Muresan M, Schaffler A, Reisch N, Schott M, Fassnacht M, Opocher G, Klose S, Fottner C, Forrer F, Plockinger U, Petersenn S, Zabolotny D, Kollukch O, Yaremchuk S, Januszewicz A, Walz MK, Eng C, Neumann HP 2009 Clinical predictors and algorithm for the genetic diagnosis of pheochromocytoma patients. Clin Cancer Res 15:6378-6385

8 Zeegers MP, van PF, Vlietinck R, Spruijt L, Ostrer H 2004 Founder mutations among the Dutch. Eur J Hum Genet 12:591-600

9 Zeegers MP, van PF, Vlietinck R, Spruijt L, Ostrer H 2004 Founder mutations among the Dutch. Eur J Hum Genet 12:591-600

10 Taschner PE, Jansen JC, Baysal BE, Bosch A, Rosenberg EH, Brocker-Vriends AH, van der Mey AG, van Ommen GJ, Cornelisse CJ, Devilee P 2001 Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes Cancer 31:274-281

11 Zeegers MP, van PF, Vlietinck R, Spruijt L, Ostrer H 2004 Founder mutations among the Dutch. Eur J Hum Genet 12:591-600

12 Erlic Z, Rybicki L, Peczkowska M, Golcher H, Kann PH, Brauckhoff M, Mussig K, Muresan M, Schaffler A, Reisch N, Schott M, Fassnacht M, Opocher G, Klose S, Fottner C, Forrer F, Plockinger U, Petersenn S, Zabolotny D, Kollukch O, Yaremchuk S, Januszewicz A, Walz MK, Eng C, Neumann HP 2009 Clinical predictors and algorithm for the genetic diagnosis of pheochromocytoma patients. Clin Cancer Res 15:6378-6385

13 Cascon A, Lopez-Jimenez E, Landa I, Leskela S, Leandro-Garcia LJ, Maliszewska A, Leton R, de l, V, Garcia-Barcina MJ, Sanabria C, Alvarez-Escola C, Rodriguez-Antona C, Robledo M 2009 Rationalization

of genetic testing in patients with apparently sporadic pheochromocytoma/paraganglioma. Horm Metab Res 41:672-675

14 Erickson D, Kudva YC, Ebersold MJ, Thompson GB, Grant CS, van Heerden JA, Young WF, Jr. 2001 Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. J Clin Endocrinol Metab 86:5210-5216

15 van Houtum WH, Corssmit EP, Douwes Dekker PB, Jansen JC, van der Mey AG, Brocker-Vriends AH, Taschner PE, Losekoot M, Frolich M, Stokkel MP, Cornelisse CJ, Romijn JA 2005 Increased prevalence of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with SDHD-linked head and neck paragangliomas. Eur J Endocrinol 152:87-94

16 Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, Keiser HR, Goldstein DS, Eisenhofer G 2002 Biochemical diagnosis of pheochromocytoma: which test is best? JAMA 287:1427-1434

17 Zak FG, Lawson W. The paraganglionic receptor system. 1982.

18 Erickson D, Kudva YC, Ebersold MJ, Thompson GB, Grant CS, van Heerden JA, Young WF, Jr. 2001 Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. J Clin Endocrinol Metab 86:5210-5216

19 van Duinen N, Steenvoorden D, Kema IP, Jansen JC, Vriends AH, Bayley JP, Smit JW, Romijn JA, Corssmit EP 2010 Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. J Clin Endocrinol Metab 95:209-214

20 Kim T, Tao-Cheng JH, Eiden LE, Loh YP 2001 Chromogranin A, an "on/off" switch controlling dense-core secretory granule biogenesis. Cell 106:499-509

21 Jain RK, Joyce PB, Gorr SU 2000 Aggregation chaperones enhance aggregation and storage of secretory proteins in endocrine cells. J Biol Chem 275:27032-27036

22 Montesinos MS, Machado JD, Camacho M, Diaz J, Morales YG, varez de la RD, Carmona E, Castaneyra A, Viveros OH, O'Connor DT, Mahata SK, Borges R 2008 The crucial role of chromogranins in storage and exocytosis revealed using chromaffin cells from chromogranin A null mouse. J Neurosci 28:3350-3358

23 Bilek R, Safarik L, Ciprova V, Vlcek P, Lisa L 2008 Chromogranin A, a member of neuroendocrine secretory proteins as a selective marker for laboratory diagnosis of pheochromocytoma. Physiol Res 57 Suppl 1:S171-S179

24 d'Herbomez M, Gouze V, Huglo D, Nocaudie M, Pattou F, Proye C, Wemeau JL, Marchandise X 2001 Chromogranin A assay and (131)I-MIBG scintigraphy for diagnosis and follow-up of pheochromocytoma. J Nucl Med 42:993-997

25 d'Herbomez M, Forzy G, Bauters C, Tierny C, Pigny P, Carnaille B, Pattou F, Wemeau JL, Rouaix N 2007 An analysis of the biochemical diagnosis of 66 pheochromocytomas. Eur J Endocrinol 156:569-575

26 Giovanella L, Ceriani L 2002 Serum chromogranin-alpha immunoradiometric assay in the diagnosis of pheochromocytoma. Int J Biol Markers 17:130-134

27 Grossrubatscher E, Dalino P, Vignati F, Gambacorta M, Pugliese R, Boniardi M, Rossetti O, Marocchi A, Bertuzzi M, Loli P 2006 The role of chromogranin A in the management of patients with phaeochromocytoma. Clin Endocrinol (Oxf) 65:287-293

28 Hsiao RJ, Neumann HP, Parmer RJ, Barbosa JA, O'Connor DT 1990 Chromogranin A in familial pheochromocytoma: diagnostic screening value, prediction of tumor mass, and post-resection kinetics indicating two-compartment distribution. Am J Med 88:607-613

29 Nobels FR, Kwekkeboom DJ, Coopmans W, Schoenmakers CH, Lindemans J, De Herder WW, Krenning EP, Bouillon R, Lamberts SW 1997 Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. J Clin Endocrinol Metab 82:2622-2628

30 Lloyd RV, Sisson JC, Shapiro B, Verhofstad AA 1986 Immunohistochemical localization of epinephrine, norepinephrine, catecholamine-synthesizing enzymes, and chromogranin in neuroendocrine cells and tumors. Am J Pathol 125:45-54

31 DeLellis RA, Lloyd RV, Eng C. Pathology and genetics: WHO classification of tumours of endocrine organs. Oxford university press. 2004.

32 Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, Buchta M, Franke G, Klisch J, Bley TA, Hoegerle S, Boedeker CC, Opocher G, Schipper J, Januszewicz A, Eng C 2004 Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 292:943-951

33 Opocher G, Schiavi F, Conton P, Scaroni C, Mantero F 2003 Clinical and genetic aspects of phaeochromocytoma. Horm Res 59 Suppl 1:56-61

34 Neumann HP, Bausch B, McWhinney SR, Bender BU, Gimm O, Franke G, Schipper J, Klisch J, Altehoefer C, Zerres K, Januszewicz A, Eng C, Smith WM, Munk R, Manz T, Glaesker S, Apel TW, Treier M, Reineke M, Walz MK, Hoang-Vu C, Brauckhoff M, Klein-Franke A, Klose P, Schmidt H, Maier-Woelfle M, Peczkowska M, Szmigielski C, Eng C 2002 Germ-line mutations in nonsyndromic pheochromocytoma. N Engl J Med 346:1459-1466

35 Gagel RF, Tashjian AH, Jr., Cummings T, Papathanasopoulos N, Kaplan MM, DeLellis RA, Wolfe HJ, Reichlin S 1988 The clinical outcome of prospective screening for multiple endocrine neoplasia type 2a. An 18-year experience. N Engl J Med 318:478-484

36 Walther MM, Reiter R, Keiser HR, Choyke PL, Venzon D, Hurley K, Gnarra JR, Reynolds JC, Glenn GM, Zbar B, Linehan WM 1999 Clinical and genetic characterization of pheochromocytoma in von Hippel-Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma. J Urol 162:659-664

37 Walther MM, Herring J, Enquist E, Keiser HR, Linehan WM 1999 von Recklinghausen's disease and pheochromocytomas. J Urol 162:1582-1586

38 Amar L, Bertherat J, Baudin E, Ajzenberg C, Bressac-de PB, Chabre O, Chamontin B, Delemer B, Giraud S, Murat A, Niccoli-Sire P, Richard S, Rohmer V, Sadoul JL, Strompf L, Schlumberger M, Bertagna X, Plouin PF, Jeunemaitre X, Gimenez-Roqueplo AP 2005 Genetic testing in pheochromocytoma or functional paraganglioma. J Clin Oncol 23:8812-8818

39 Gagel RF, Tashjian AH, Jr., Cummings T, Papathanasopoulos N, Kaplan MM, DeLellis RA, Wolfe HJ, Reichlin S 1988 The clinical outcome of prospective screening for multiple endocrine neoplasia type 2a. An 18-year experience. N Engl J Med 318:478-484

40 Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, Keiser HR, Goldstein DS, Eisenhofer G 2002 Biochemical diagnosis of pheochromocytoma: which test is best? JAMA 287:1427-1434

41 Havekes B, van der Klaauw AA, Weiss MM, Jansen JC, van der Mey AG, Vriends AH, Bonsing BA, Romijn JA, Corssmit EP 2009 Pheochromocytomas and extra-adrenal paragangliomas detected by screening in patients with SDHD-associated head-and-neck paragangliomas. Endocr Relat Cancer 16:527-536

42 Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, Buchta M, Franke G, Klisch J, Bley TA, Hoegerle S, Boedeker CC, Opocher G, Schipper J, Januszewicz A, Eng C 2004 Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 292:943-951

43 Pomares FJ, Canas R, Rodriguez JM, Hernandez AM, Parrilla P, Tebar FJ 1998 Differences between sporadic and multiple endocrine neoplasia type 2A phaeochromocytoma. Clin Endocrinol (Oxf) 48:195-200

44 Walther MM, Reiter R, Keiser HR, Choyke PL, Venzon D, Hurley K, Gnarra JR, Reynolds JC, Glenn GM, Zbar B, Linehan WM 1999 Clinical and genetic characterization of pheochromocytoma in von Hippel-Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma. J Urol 162:659-664

45 Walther MM, Herring J, Enquist E, Keiser HR, Linehan WM 1999 von Recklinghausen's disease and pheochromocytomas. J Urol 162:1582-1586

46 Waguespack SG, Rich T, Grubbs E, Ying AK, Perrier ND, yala-Ramirez M, Jimenez C 2010 A Current Review of the Etiology, Diagnosis, and Treatment of Pediatric Pheochromocytoma and Paraganglioma. J Clin Endocrinol Metab

47 Havekes B, van der Klaauw AA, Hoftijzer HC, Jansen JC, van der Mey AG, Vriends AH, Smit JW, Romijn JA, Corssmit EP 2008 Reduced quality of life in patients with head-and-neck paragangliomas. Eur J Endocrinol 158:247-253

48 Piruat JI, Pintado CO, Ortega-Saenz P, Roche M, Lopez-Barneo J 2004 The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia. Mol Cell Biol 24:10933-10940

49 Pack AI, Gislason T 2009 Obstructive sleep apnea and cardiovascular disease: a perspective and future directions. Prog Cardiovasc Dis 51:434-451

Curriculum Vitae

Nicolette van Duinen werd geboren op 17 juni 1981 te Dordrecht. Zij behaalde haar VWO diploma in 2000 aan het Insula College te Dordrecht. In 2000 werd ze ingeloot voor de studie geneeskunde aan de Universiteit Leiden. In 2007 verrichte zij haar wetenschapsstage op de afdeling endocrinologie van het Leids Universitair Medisch Centrum. Onder begeleiding van Monique Wassenaar deed zij onderzoek naar gewrichtsklachten bij patiënten met acromegalie. Na het behalen van het artsexamen in2008 startte zij met promotie-onderzoek op de afdeling Endocrinologie van het Leids Universitair Medisch Centrum. Onder leiding van Professor dr. J.A. Romijn, Prof. Dr. J.W.A. Smit en Dr. E.P.M. van der Kleij-Corssmit heeft zij wetenschappelijk onderzoek verricht naar paragangliomen. De resultaten van dit onderzoek staan beschreven in dit proefschrift.
Sinds januari 2011 is zij werkzaam als arts-assistent in opleiding tot internist in het HagaZiekenhuis te Den Haag.

List of publications

Wassenaar MJE, Biermasz NR, van Duinen N, van der Klaauw AA, Pereira AM, Roelfsema F, Smit JWA, Kroon HM, Kloppenburg M, Romijn JA. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly: a case-control study. European Journal of Endocrinology, 2009 Mar;160 (3):357-65

van Duinen N, Steenvoorden D, Kema IP, Jansen JC, Vriends AHJT, Bayley JP, Smit JWA, Romijn JA, Corssmit EPM. Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. Journal of Clinical Endocrinology 2010 Jan; 95 (1): 209-214.

van Duinen N, Steenvoorden D, Bonsing BA, Vuyk J, Vriends AHJT, Jansen JC , Romijn JA, Corssmit EPM. Pheochromocytomas detected by biochemical screening in predisposed subjects are associated with a lower prevalence of clinical and biochemical manifestations and smaller tumors than pheochromocytomas detected by signs and symptoms. European Journal of Endocrinology 2010

van Duinen N, Kema IP, Romijn JA, Corssmit EPM. Plasma chromogranin A levels are increased in a small portion of patients with head and neck paragangliomas. Clinical Endocrinology 2010

Hensen EF, van Duinen N, Jansen JC, Corssmit EPM, Romijn JA, Vriends AHJT, van der Meij AGL, Cornelisse CJ, Devilee P, Bayley JP. High prevalence of founder mutations in genes causing hereditary paraganglioma and pheochromocytoma syndrome in the Netherlands. Clinical Genetics 2011 Feb 23 [Epub ahead of print]

Biermasz NR, Joustra SD, Donga E, Pereira AM, van Duinen N, van Dijk M, van der Klaauw AA, Corssmit EP, Lammers GJ, van Kralingen KW, van Dijk JG, Romijn JA. Patients previously treated for nonfunctioning pituitary macroadenomes have disturbed sleep characteristics, circadian movement rhythm, and subjective sleep quality. J Clin Endocrinol Metab. 2011 May;96(5):1524-32. Epub 2011 Mar 2.

Havekes B, Kastelein F, van der Klaauw AA, van Duinen N, Jansen JC, Smit JW, van Kralingen KW, Vriends AH, Romijn JA, Corssmit EP. Head-and-neck paragangliomas are associated with sleep-related complaints, especially in the presence of carotid body tumors. Sleep Breath. 2011 May 27. [Epub ahead of print]

van Duinen N, Corssmit EPM, de Jong WHA, Brookman D, Kema IP, Romijn JA. Plasma levels of free metanephrines and 3-methoxytyramine indicate a higher number of biochemically active HNPGL than 24 h urinary excretion rates of catecholamines and metabolites. Submitted for publication

van Duinen N, van Kralingen KW, van Dijk JG, Ninaber MK, Havekes B, Smid L, Lammers GJ, Jansen JC, Smit JW, J.A. Romijn, E.P.M. Corssmit. High prevalence of sleep disordered breathing in patients with bilateral carotid body tumors is associated with increased peripheral chemoresponsiveness. Submitted for publication

van Hulsteijn LT, van Duinen N, Romijn JA, Smit JWA, Corssmit EPM. Urinary 5-HIAA levels in patients with head and neck paragangliomas. Submitted for publication